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CAVITATION AND XYLEM RESISTANCE IN CUCURBITA PEPO

by

DANIEL R. THOMPSON

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Cavitation and Xylem Resistance in *Cucurbita pepo*", submitted by Daniel R. Thompson in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Recent investigations have detected breakage of water columns, or cavitation, in the xylem of herbaceous and woody plants and suggested that such events occur regularly when xylem tensions are high. If enough water columns cavitate, xylem resistance may increase. This problem was investigated using squash (*Cucurbita pepo*).

Squash has an efficient long-distance water transport system, due mainly to large radius vessels and simple perforation plates. Also, its tracheary elements are apparently relatively short, and may thus prevent a spread of cavitations through an entire plant. Stem and petiole transverse sections contained large numbers of tracheary elements, so even large numbers of cavitations would be unlikely to completely stop long-distance transport.

In order to detect cavitations in intact plants, the driving force for water flow was maintained constant and water flow was measured during treatments designed to stimulate cavitation. To assure that changes in either root or leaf resistance would not be confused with changes in xylem resistance, steps were taken to eliminate such changes or, if impractical, to quantify them. Two treatments were undertaken: severing of the xylem in a step-wise fashion at one point along the stem, and elevation of tension by osmotic treatments. Severing 10 of 12 vascular bundles or osmotic treatments to produce xylem tensions as low as -4.0 MPa (-40 bars) did not produce any measurable change in xylem resistance.

Unfortunately, the experimental design allowed for little measurement sensitivity to changes in xylem resistance, so xylem resistance may actually have increased during the osmotic treatment, as it did in the cutting treatment.

Even though xylem resistance could not be shown to change in these experiments, it was suggested that such a change must occur to some extent. Since the driving force necessary for flow in the xylem of squash is so small and the conductive capacity is so high, any such changes in xylem resistance usually have little impact upon the physiological processes of the leaves. In some situations, such as during rehydration after a period of water deficit, increased xylem resistance may play a role by delaying the return of physiological processes to normal. Studies of the vascular morphology of squash and other plants should consider the possibility of selection to minimize the effect of cavitation.

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INTRODUCTION

The exploration of properties and characteristics of water flow in the xylem of plants has been under study since 1727 when Stephen Hales published his pioneering work Vegetable Statics. Some aspects of the physics of xylem flow as well as the evolutionary and mechanical roles of the xylem are still not well understood (Carlquist 1975). This is due to inability to study them in an undisturbed and isolated state. Since conduction in the xylem is predominantly not within living cells, characteristics of flux within the xylem may be partially described by physical laws.

Only the xylem can supply aerial portions of an plant with water and essential minerals. It is imperative that continuity of water columns within the xylem remain, as the tension-cohesion theory indicates it must. Recent experimental and theoretical work suggests that the continuity of at least some of the water columns in the xylem may be regularly lost due to the development of high xylem tensions. Milburn and Johnson (1966) dramatically demonstrated this by acoustically detecting the small vibrations which occurred as water columns cavitated. Earlier workers have detected cavitation directly by visual means (Crafts 1939; Stocking 1943). Jarvis (1974), Hsaio (1973), and Newman (1976) have all recently pointed out that there is much opportunity to investigate the effects of cavitation on water flux and to further assess the physiological implications of such a modified flow. Only indirect methods involving some destruction of the xylem or extreme treatments (e.g.: liquid nitrogen freezing) have been used to attempt to solve this

problem. They must be interpreted with great caution for this reason (Greenridge 1955; Byrne _et al_. 1977).

The purpose of the work undertaken was to determine quantitatively the effect of cavitation on xylem resistance to water flow in an intact plant system. Specifically, the work would determine: 1) if mid-day stresses which a plant may normally encounter would be sufficient to cavitate water columns and change xylem resistance on a diurnal basis, and 2) the relation between tension, cavitation, and xylem resistance during abnormally high xylem tensions.

Simplification of the experimental design was deemed necessary to isolate the xylem from the rest of the plant, experimentally and mathematically. Resistance may be determined by controlling the driving force for water flux in the xylem and directly measuring the water flow. By maintaining the same magnitude of driving force and increasing water deficit, tension in the xylem can be increased. Cavitation and an increase in resistance may occur and be apparent as a decrease in water flow. The advantage to this approach is that it avoids measurement of the driving force, which is difficult to do accurately.

Initially, an experimental system was sought which would depend entirely upon liquid water exchange through roots and the xylem, and thus would be characterised by high rates of flux and a great sensitivity to changes in xylem resistance. Unfortunately, the systems devised proved unfeasible. A second type of design utilized an additional pathway, a vapor pathway. The characteristics of this system precluded high rates of flux and involved a low

sensitivity to changes in xylem resistance. Further explanations describing aspects and limitations of this design will be presented.

Squash (Cucurbita pepo) was chosen for the experiments for four reasons: 1) Qualitatively, it has been shown to experience cavitations (Crafts et al. 1949, p. 160; Stocking 1943), 2) it has high rates of water flux, 3) its xylem contains very large vessels which are are believed to cavitate more readily than small vessels, and 4) it is easily grown in solution culture.

Studies were designed to characterize the vascular morphology of squash in order to look for relations between vessel anatomy, flow, and cavitation. Changes in xylem resistance were sought during two experimental treatments: artificial breaking of xylem conduits and imposing high tensions on water columns. In order to provide familiarity with the water relations of the plant, an ancillary study was conducted to measure several water status indicators during dehydration-rehydration cycles.

LITERATURE REVIEW

DRIVING FORCE FOR WATER TRANSPORT

Water moves from the soil to the air through plants in response to a gradient in the free energy of water along the pathways of movement. The amount of water transported is dependent upon the resistance of the pathway, as well as the steepness of the gradient of free energy. Van den Honert (1948) applied these concepts and formulated the Ohm's law analogy of water flux in plants:

$$J = \frac{\psi_1 - \psi_2}{R} \qquad (1)$$

where: J is the volume flux of water per unit time, ψ_1 and ψ_2 are the free energies or water potentials of any two arbitrary points along the pathway, and R is the resistance to water movement associated with the pathway between the arbitrary points along the pathway. Assuming steady state conditions, equ. 1 may further be enlarged to give:

$$J = \frac{\psi_s - \psi_r}{R_s + R_r} = \frac{\psi_r - \psi_l}{R_x} = \frac{\psi_l - \psi_a}{R_l + R_a} \qquad (2)$$

where subscripts s, r, x, l, and a refer to the substrate (soil or nutrient solution), the living cells of the root, the xylem, the interior and surface of the leaf, and the air, respectively. Richter (1973) reviewed the various forms of equ. 2 presented in the literature, and suggested that in order to account for branching and to provide the correct dimensions, van den Honert's formulation be modified to give:

$$\psi_p = \psi_s - \sum_{i=s}^{p} J_i R_i \quad , \qquad (3)$$

where the subscript p refers to any arbitrary point. Although Richter's modification is dimensionally and physically correct, equ. 2 is a more useful model when dealing with fluxes.

PATHWAYS OF WATER TRANSPORT

Soil. Water movement from the soil to plant roots is classified as perirhizal and pararhizal (Jarvis 1976). Perirhizal movement occurs in the zone of soil surrounding the root, and pararhizal movement occurs as water is transported from one part of the soil mass to another along gradients in soil matric potential. In saturated soils, neither of these pathways present appreciable resistance to water movement. In unsaturated soils, resistances to water movement in both pathways may increase greatly (Newman 1969; Jarvis 1974). In the case of perirhizal movement, resistance may increase as the roots dehydrate and shrink and vapor gaps are formed. Pararhizal resistance increases as hydraulic conductivity of the soil decreases.

Root. Several investigators have shown that removal of root systems brings about an increase in transpiration rates, suggesting that roots account for a large portion of resistance within a plant (e.g.: Jensen et al. 1961). Boyer (1969, 1971a) found that for bean, sunflower, and soybean, the root system accounted for 45, 50, and 70% respectively, of the total plant resistance when the stomata were open. Although the relative magnitude of root resistance is known for at least some species, the pathway of water as it moves

from the soil to the root xylem is still in question. Newman (1976) listed these as: 1) the vacuolar pathway, in which water travels through the cortex by passing through vacuoles, 2) the free space-endodermis pathway, in which transport occurs through cortical cell walls and protoplasts at the epidermis, 3) the symplasm pathway, in which water moves across the cortex in plasmodesmata. There is not enough experimental evidence to state which pathway has the least resistance and is thus primarily responsible for conduction. Newman (1976) has calculated for maize that the most likely is the symplasm pathway.

The region near the root tip is most effective in absorption of water. Generally, most absorption occurs 5-10 cm behind the tip (Slatyer, p. 200); beyond 10 cm, superficial suberization occurs and hinders water uptake. For squash, Graham, Clarkson, and Sanderson (1974, cited in Newman, 1976), found greatest water uptake occurring 2-10 cm from the root tip, but reduced uptake occurred until at least 26 cm from the root tip. Even though suberization generally does inhibit uptake, there may be exceptions. Brouwer (1953) found that root permeability was highest in the younger parts of broad bean root systems when the rate of water uptake was low, while older, more suberized portions had the highest permeability when the uptake rate was elevated. Furthermore, Chung and Kramer (1975) have shown that woody species can absorb water through suberized roots. They suggested that such absorption plays a major role in the overall water relations of such species.

Parsons and Kramer (1974) studied root resistance as a function of time of day and applied pressure. They found that it varied on

a diurnal cycle with the greatest resistance (2 - 3 times greater) occuring at midnight. They attributed the changes to variations in ion permeability, hormones, or root growth. Coupled solute and water flux may also account for this phenomenon (Fiscus 1975; Dalton *et al*. 1975).

Xylem. Long distance conduction of water through the root, stem, and leaf xylem occurs in tracheary elements. Since they behave approximately as ideal capillaries, flow through them is describable by the Hagen-Poiseuille law:

$$J = \frac{\pi\ \Delta p\ \Sigma(r^4)}{8\eta\ell} \quad , \tag{4}$$

where Δp is the hydrostatic pressure gradient, η is the viscosity of water, ℓ is the length of the conducting section, and $\Sigma(r^4)$ is the sum of the radii to the fourth power of the conducting capillaries. When equ. 4 is applied to one plant or part thereof, and temperature remains constant (so η does not change), equ. 4 reduces to:

$$J = \frac{\Delta p}{k\,\Sigma(r^{-4})} \quad , \tag{5}$$

where k is a constant. The form of this equation is similar to equ. 1 and shows the importance of the $\Sigma(r^4)$ term in determining xylem resistance. Water moves through capillaries at graded velocities: molecules adjacent to the wall are stationary; those in the center move the fastest. The peak velocity for a single capillary is (Zimmermann 1971, p. 190):

$$U = \frac{\Delta p\ r^2}{4\ell\eta} \quad , \tag{6}$$

where U is the peak velocity.

The Hagen-Poiseuille law only approximates description of the hydraulic characteristics of the xylem. Although some vines have experimentally been found to give conductivities equal to calculated values, many species give experimental values from 10 - 80% of the calculated values. Zimmermann (1971, p. 196) suggests that limited capillary length is the most important factor in decreasing conductivity. This factor is most important when dealing with tracheids which are usually only a few millimeters in length. However, since little water flow normally occurs through tracheids in plants with vessels, short length is not a critical factor in that case. Length of vessels is rather difficult to determine, but has been found to be from 60 to nearly 600 cm (Esau 1965, p. 229). For Gossypium hirsutum, two different populations of vessels were found, one about 5.5 cm and another about 16 cm in length (Mackay and Weatherly 1973). In vessels where perforations are incomplete, additional resistance to flow may be found. The fact that xylem walls are not smooth has no effect, since rates equal to theoretical have been found in certain vines (Zimmerman 1971, p. 198). Lack of perfect roundness in cross-section does not bring about major deviations either (Preston 1938).

The relative magnitude of xylem resistance in relation to other resistances is small. Few measurements are available, but Boyer (1971a) has found xylem resistances to account for 25, 22, and 7% of the total plant resistance in transpiring sunflower, bean, and soybean respectively. Jarvis (1974) has listed several other studies which attempted to measure xylem resistance. From equ. 4,

it is easily seen that length of the xylem plays an important role. For this reason it is difficult to compare studies of trees and herbs.

The most difficult aspect of dealing with equ. 5 is estimating $\Sigma(r^4)$. It cannot be assumed that all the capillaries will be conducting. It is well known that the content of air in tree stems increases during a growing season (Greenridge 1957), indicating some conducting elements have been blocked by air, or embolized. Heartwood or tylose formation, or both, can often occur following embolism, thereby permanently removing conductive capacity of a vessel or tracheid.

Cavitation of an existing water column is the first step in the blockage of a tracheary element. Water in tracheary elements is usually subjected to a negative pressure, or tension, due to transpirational pull and is thus in a metastable state. If the hydrostatic pressure decreases to a point beyond the tensile strength of water, the continuous column of water breaks and the conductive continuity is lost. Since Askenasay, Dixon, and Joly advanced the tension-cohesion theory of sap ascent in 1895, there have been many estimates of the tensile strength of water. As Table 1 shows, there is a wide range of values given, dependent upon the particular technique used. Generally, experimental methods have given the lowest strengths. Milburn and Johnson (1966) developed an in situ technique for detection of cavitation in intact plant tissues. Apparently, a small "click" is emitted when a water column cavitates and it may be detected by a sensitive acoustic amplifier. This technique should be considered with caution, but has led Milburn

Table 1. Tensile strength of water as determined by several methods. 1 MPa = 10 bars.

Tensile Strength (MPa)	Technique	Date	Author
1500	calculation	1948	Newman and Seale
135	calculation	1948	Fisher
45-80	calculation	1947	Temperly
30	fern annulus	1954	Haider
30	fern annulus	1944	King
0.4-60	steel plate	1912	Budgett
10	superheating	1924	Kenrick et al.
20	Berthelot tube	1914	Dixon
3.4	Berthelot tube		Meyer (see Greenridge 1957)
3.2	Berthelot tube	1946	Temperly
3.0	Berthelot tube	1948	Scott et al.
2.5	Berthelot tube	1960	Loomis et al.
3.0	dye injection	1955	Greenridge
27	centrifugation (22°C)	1950	Briggs
1.0	centrifugation (1°C)	1950	Briggs
>1.7	centrifugation	1955	Scholander
0.5	acoustic	1974	Milburn and Mclaughin
1.2	acoustic	1976	West and Gaff
2.0	leaf water potential	1971b	Boyer

(1973a, 1973b), Milburn and Mclaughin (1974), and West and Gaff (1976) to suggest that cavitation occurs quite readily even under relatively small hydrostatic tensions. Milburn and Mclaughin (1974) further suggested that cavitation may occur on a regular diurnal basis when transpirational demand is high. It is not clear if the cavitated columns of water may reform while the water in the xylem is still experiencing a tension. Water supplied by other capillaries may be able to refill cavitated elements (Crafts 1939; Meidner and Sheriff 1976, p. 64). It is unknown how long is required for cavitated elements to become air filled. Since cavitations are restricted to a single capillary due to the surface tension of water at pit connections between tracheary elements, air cannot directly enter the cavitated element, and the lengthy process of diffusion through cells must occur (Zimmermann 1971, p. 207).

Cavitation events are probably precipitated from nucleation by pre-existing microbubbles adhering to cell walls (Hayward 1971). Since large vessels naturally have more area of cell wall adjoining water, they would be expected to cavitate more often. Good experimental evidence on this point is lacking, but Crafts (1939) did show greater susceptibility to cavitation in larger vessels. Since volume flux is proportional to the fourth power of the capillary radius, cavitation of a large capillary would have a much greater effect upon xylem resistance than cavitation of a very small capillary.

Temperature may modify the ease with which cavitation occurs. Briggs (1950) found water to have a tensile strength of over 27 MPa from 4 - 50°C, but the tensile strength decreased to about

only 1.0 MPa at 1°C. Based upon this evidence, cavitation may play an even more important role during low, but above freezing temperatures. Freezing temperatures may bring about cavitation because air has a very low solubility in ice. Here again, there is a relation between conducting element diameter and exclusion of cavitation; conifer tracheids and smaller vessels can apparently reform continuous water columns upon thawing, while large angiosperm vessels cannot (Hammel 1967).

The morphology and development of the xylem is related to the occurence of cavitations. For instance, long vessels are more efficient conductors, but shorter ones are safer since surface tension of water at pits will not allow cavitations to spread from one vessel to another (Zimmermann 1971, p. 207). Lateral transport through pits and rays (Mackay and Weatherly 1973) and fusion of vascular strands at nodes (Diamond 1966) provide for alternate pathways in the event of cavitation. It appears that vascular systems may have evolved some structural modifications in response to cavitation. This allows retention of conductive capacity even if cavitations occur regularly.

Secondary growth is an important way in which continuous water columns are maintained. At maturity tracheary elements are full of water. They thus serve to restore and increase conductive capacity. In monocotyledons and certain dicotyledons which show little or no secondary growth, root pressure is often very common and may be an alternate strategy of maintaining continuous water columns. Root pressures of 0.1 to 0.2 MPa have been commonly observed (Slatyer 1967, p. 210), and occasionally have been noted to be as high as 0.8 MPa (Kramer 1949, p. 210). Gravitational

forces as well as xylem resistance must be overcome to allow water to be carried to the tops of plants by root pressure, but a root pressure of 0.1 MPa could probably pump water nearly 10 m high, presumably refilling cavitated and embolised tracheary elements in the process.

Leaf. The main pathways for water movement in the leaf are the vascular bundles of the veins. Slatyer (1967, p. 215) has pointed out that this distribution system is very effective; only rarely are leaf cells separated by more than two other cells from a vascular element. To ensure that air is not allowed into the vascular system, living cells are located around the end of a vascular bundle. Water moves from the xylem through pits and cell walls into the cell walls of adjacent cells. Evaporation or further liquid phase transport, or both, may then occur. Nearly all of the water transpired from leaves exits through the stomata, but a measurable amount is evaporated through the cuticle as well. Generally, the amount of transpiration occurring through the cuticle is very small--cuticular resistances have been found to be from 20-200 s/cm for a number of species (Slatyer 1967, p. 261). However, Aston and Jones (1976) have suggested that some of these estimates are too low. Meidner (1975) has stated that cuticular transpiration is only about 3% of stomatal.

The actual pathway of liquid water transport within the mesophyll and epidermis is not yet proven. The classical view from tracer studies is that most water transport and evaporation occurs in the mesophyll cell walls. Water vapor then escapes via the substomatal cavities and stomatal openings. Several recent

studies have presented convincing evidence that the mesophyll cell walls may not be associated with as much transport and evaporation as formerly thought. Meidner (1975) found that an isolated epidermis of Tradescantia virginiana could transpire as much water as an intact leaf, indicating high hydraulic conductivity in the epidermis. A number of studies using a variety of tracer techniques have generally shown that a great deal of transpiration occurs from the inner walls of guard, subsidiary, and adjacent epidermal cells, and that the epidermis may be a primary pathway (Byott and Sheriff 1976; Aston and Jones 1976; Burbano et al. 1976; Pizzolato et al. 1976). All of these studies suggest that evaporation from the mesophyll is equal or smaller than that from other cells. Results of such tracer studies have been validated by Meidner (1976) who found that the greatest evaporation would occur from nearest the pore in a scale model of a substomatal cavity. This suggested that purely on a physical basis, greatest evaporation would be expected from the inner wall of a guard cell.

The redevelopment of theories of water transport in leaves may also force investigators to re-examine the so-called mesophyll resistance to transpiration, which has been suggested to increase as dehydration occurs (Slatyer 1966, p. 257; Cox and Boersma 1967; Jarvis and Slatyer 1970). Conclusive detection and measurement of mesophyll resistance changes on intact and undamaged leaves is lacking.

As stated earlier, a very small amount of water is lost through the outer epidermal walls and cuticle. According to Slatyer (1967, p. 220) the liquid-air menisci probably occur at the waxy layer and

outer epidermal wall interface. Thus the wax layer may be effective in decreasing vapor phase transport as well as liquid phase transport. Aston and Jones (1976), using Si as a tracer, obtained results which suggested that cuticular transpiration in barley occurs mainly from exposed anticlinal epidermal cell walls and that higher rates may be expected from epidermal fiber cells above the veins.

Cuticular resistance is also affected by environmental factors. Moreshet (1970) showed that low relative humidity stimulated high cuticular resistance. This property is undoubtedly related to Schönherr's (1976) finding that water permeability of the cuticle is directly proportional to its water content. Holmgren *et al.* (1965) showed cuticular resistance was affected by temperature, as well. A temperate zone species, *Betula verrucosa*, had a cuticular resistance of about 50 s/cm at 22°C, but was about 140 s/cm at 17°C.

Air. The final resistance to water movement through the soil-plant-atmospheric continuum results from the formation of an unstirred layer at the surface of the leaf. This resistance, the boundary layer resistance, is a function of the thickness of the unstirred layer. This, in turn is a function of the leaf dimensions and wind velocity. In general, boundary layer resistance is small in comparison to stomatal resistance in wind velocities normally encountered. Boundary layer resistance may be calculated easily from simple equations (Nobel 1970, p. 305).

METHODS AND MATERIALS

PLANT CULTURE

Seeds of hybrid squash, Cucurbita pepo cv "Baby Crookneck", were obtained from Stokes Seed Company (St. Catherines, Ontario) and germinated in vermiculite in a greenhouse. When the seedings were at least 10 cm high and the cotyledons were fully expanded (usually requiring 7 - 10 days) they were transferred to 2 l plastic pots containing full strength Hoagland's solution (Epstein, 1972). The solutions were continuously aerated from building compressed air. The plants were then grown for up to 4 weeks in the greenhouse. Temperature was maintained at building temperature, usually 22°C, but varied from 21 - 24°C. Relative humidity (RH) was not controlled, but was usually around 30%. During the summer natural lighting was sufficient, but during winter supplemental lighting from Multivapor and Lucalux lamps (Sunbrella, Environmental Growth Chambers, Chagrin Falls, Ohio) supplied about 500 $\mu E/m^2$-s of photosynthetically active radiation at the top of the canopy. The photoperiod was 14 h to encourage vigorous growth and to discourage flowering.

The amount of nutrient solution was kept constant by watering daily with distilled water. A complete renewal was made every 10 days. Plants which had been growing in Hoagland's solution for approximately 2 or 4 weeks were used, depending on the experiment. Plants which had been in culture for 2 weeks usually had 3 or 4 leaves which were only partially expanded. The older plants had 8 - 11 leaves, most of which were fully expanded. At least 4 days

before a plant was to be used, it was transferred to a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio). The photoperiod length was kept the same as in the greenhouse, but the light and dark periods were reversed so that experiments could be performed during the daytime on plants pretreated in the dark. Temperature and relative humidity were maintained continuously at 22°C and 35 ±5%, respectively. Light was from a mixture of fluorescent and incandescent bulbs, giving a photosynthetically active radiation of approximately 180 $\mu E/m^2$-s at the top of the canopy.

VESSEL RADII AND FREQUENCY DISTRIBUTION

Stem and petioles. A plant which had been in solution culture for 4 weeks was used to make freehand sections for determination of the number of vessels and their radii. The midpoints of 3 mature internodes and each petiole were sampled, stained briefly for lignin with basic fuchsin, mounted in water and their vessels were counted and measured. A micrometer eyepiece calibrated from a standard slide (Erma, Tokyo) was used on a Reichert microscope (Austria), giving a resolution of 0.1 μm at 400X. Since the vessels were typically ellipsoid in shape, the longest and shortest inside dimensions were recorded for later calculations. No attempt was made to positively identify the smaller cells (inside radius: 5 - 10 μm) in the xylem which had lignified walls to be perforate or imperforate. Due to their very small diameters, they are not important in water transport if they were perforate. The collected data were transferred to computer cards and conversions, ranking, and other calculations were done by a PL/I computer

program at the University of Alberta computing center. Preston's (1938) modification of the Hagen-Poiseuille law (equ. 4, p. 7) was used in all calculations to correct for ellipsoidal capillaries. It simply requires that r in equ. 4 be replaced by the square root of the product of the radii of the two ellipsoid axes.

Roots and leaf blade. Due to the difficulty encountered in freehand sectioning of root and leaf tissue for microscopic observation, these tissues were fixed, dehydrated, embedded in paraffin, sectioned, and stained for observation. Procedures generally followed that given by Jensen (1962). Leaf tissue was obtained from a mature leaf and included major veins, but not the midrib. Root tissue was taken from the root tips, from approximately midway along a 60 cm primary root, from very near (5 - 8 cm) the root collar, and finally, from the smallest roots which could be handled.

The tissue was fixed in formalin-acetic acid-alcohol (FAA) for 24 h, then dehydrated in an ethyl alcohol-tertiary butyl alcohol series. When dehydration was completed, the samples were infiltrated and embedded in paraffin. The sections were cut with a rotary microtome and the ribbons affixed to slides with Haupt's adhesive. The slides were next deparaffined with xylene and an absolute alcohol series, then the tissue was partially hydrated in an alcohol series. Lignin was stained with ammoniacal basic fuchsin and the alcohol series reversed to dehydrate the tissue. Cellulose walls were then stained with fast green and differentiated in a mixture of 50% clove oil, 25%

absolute alcohol, and 25% xylene. Finally, the slides were rinsed in xylene, and the cover slips were mounted with balsam. Vessels were then counted and measured by the same method as with stems and petioles.

VESSEL HYDRAULIC CONDUCTIVITY

The efficiency of water conduction in squash was determined by comparing actual measured conduction with the theoretical value calculated by the Hagen-Poiseuille law. Five plants which had been grown in Hoagland's solution for 4 weeks were used. A short segment (1.5 - 3.8 mm) of one vascular bundle was dissected from the hypocotyl along with 1 mm of surrounding tissue. A rubber sleeve was attached to the segment, filled with water and the free end was attached to a 100 ml burette containing boiled distilled water. All dissection and most preparation was done with the tissue immersed in boiled distilled water to aid in the prevention of embolism. The burette was opened and water was allowed to run freely through the vessels for about 30 seconds. Measurements were then made by noting the time required for 2.0 ml of water to run through the bundle. The average height of the water column was recorded to calculate the hydrostatic pressure. Three determinations of the rate of water flux were made on segments from each of the five plants used to give actual measured conduction.

After the above determinations, vessel sizes and frequencies were determined (using the same methods as for stems) at 3 - 5 places along the hypocotyl segment. With these vessel measurements, the hydrostatic pressure, the length, and the viscosity of

absolute alcohol, and 25% xylene. Finally, the slides were rinsed in xylene, and the cover slips were mounted with balsam. Vessels were then counted and measured by the same method as with stems and petioles.

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water at 22°C, the theoretical rate of conduction was calculated from equ. 4 (p. 7) using a computer program. Comparison was then made to the value obtained by actual measurement, as given above.

MEASUREMENT OF RESISTANCE TO WATER TRANSPORT

Theoretical aspects. Equation 2 (p. 4) showed that in a steady state, the flux of water through each pathway (roots, stem, and leaves) is equal and proportional to the difference in water potential along the respective pathways. This equation may be shortened to give:

$$J = \frac{\psi_s - \psi_a}{R_s + R_r + R_x + R_l + R_a} \qquad (7)$$

where the symbols are as before. If $\Delta\psi_{s-a}$, R_s, R_r, R_l, and R_a are held constant (or change by a known amount) any change in J beyond that expected due to those resistances (in response to a treatment) must be due to a change in R_x. If ψ_a is very small, say -313 MPa (-3130 bars, corresponding to RH = 10% at 22°C and ψ_s is never less than -5.0 MPa, $\Delta\psi_{s-a}$ is effectively constant. R_s is quite negligible in solution culture and R_a can be maintained effectively constant and very small in an atmosphere of turbulent air. This accounts for all but R_r and R_l. If the plant is kept in the dark, however, the stomata will be closed, eliminating a major source of a changing resistance in the leaves. Properties of the cuticle and also of the evaporative pathways in the leaves may change in response to time or water deficit, or both, thus leading to changes in R_l. Root resistance may also be affected by time or water deficit. Experiments described below will examine these possibilities, as well as attempt to detect changes in R_x.

Root resistance. Root resistance was measured on plants which had been grown in solution culture for 2 weeks. After 4 - 5 h into their normal dark period, shoots were removed from the plants with a razor blade and the root systems were placed in distilled water in a 200 ml plastic container. The transfer was done in about 10 s. The container was then placed in a Scholander pressure chamber and a rubber sleeve was attached to the stem at the cut surface and to a glass tube sealed into a rubber stopper in the pressure chamber lid. The rubber sleeve and glass tube assembly was then filled with distilled water, the pressure chamber was sealed, and the protruding glass attached to a pipette for measuring the rate of water uptake. Usually, the volume of water measured (which was timed with a stopwatch) was 0.100 ml, but could be reduced to as low as 0.020 ml or as great as 0.500 ml, depending upon the rate of water flux and the degree with which it changed.

Using this system, three kinds of data were collected: the rate of water output due to root pressure, the effect of hydrostatic pressure on water output, and the effect of dehydration upon water output. When output due to root pressure was measured no additional treatment was necessary. The effect of hydrostatic pressure was determined by applying a pressure from compressed air into the chamber and measuring the resulting output. Finally, the effect of dehydration on output was determined by first establishing the output rate for a given pressure, then replacing the distilled water around the roots with osmoticum of equal (but oppositely signed) osmotic pressure to the hydrostatic pressure used. The chamber

was then pressurized to twice the original pressure. If there was no change in root resistance, the same rate of water output would be expected.

NaCl was used as an osmoticum because it is soluble at the concentrations needed for these and other experiments. Following the recommendation of Levitt (1969, p. 68), $CaCl_2$ was added to the solutions so that, on a molar basis, there was 10 times as much Na^+ as Ca^{++}. The Ca^{++} apparently is necessary to inhibit the uptake of Na^+ by protoplasts. Solutions of the above reagents of known osmotic potentials were made according to Lang (1967) for NaCl and Levitt (1969, p. 62) for $CaCl_2$.

Leaf Resistance. Leaf resistance in the dark as a function of leaf water potential was studied by measuring weight loss of a detached section of leaf as it dried in a darkened chamber of low humidity. It should be noted that this method does not necessarily reflect what occurs in an intact leaf. The normal supply of water to the leaf has been cut off, thereby forcing transpirational water to be extracted from other than normal sources.

Mature leaf tissue from plants grown in solution for approximately four weeks was used in these experiments. The plants had been kept in the growth chamber for several days before the experiment and had entered their normal dark period about 5 h before the start of the experiment. An entire leaf was cut from a plant and moved to a darkened laboratory from the growth chamber. From the leaf, a rectangular piece of tissue about 4 by 6 cm was quickly cut out, taped to a small wire hanger and suspended in a Mettler H10 analytical balance. Very dry air

(RH = 4-5%) from building compressed air supply was injected into the balance chamber at a rate of approximately 5.5 l/min. Windspeed inside the chamber was assumed to be constant. A small outlet tube connected to a vacuum removed a sample of air for periodic checks on relative humidity and temperature. Relative humidity and temperature remained within ± 1% and ± 1^{o}C, respectively, within a single experiment, although values differed between experiments, depending upon atmospheric conditions. Either a dew point hygrometer (E, G & G, model 880, Cambridge, Massachusetts) or a calibrated electric hygrometer (Model 15-3001, Hydrodynamics, Inc., Silver Springs, Maryland) gave a continuous reading of dew point or relative humidity, respectively.

Rate of water loss was measured by momentarily turning off the input air and weighing the tissue to 0.0001 g. During the first hour of the experiment the tissue changed weight rapidly, so weighing was done at 2 min intervals. As weight changes became less dramatic, weighings at 30-60 min intervals were made for up to 12 h.

Periodically, 5 mm diameter leaf discs were removed from the drying tissue and their leaf water potential measured using thermocouple psychrometers, as outlined in Appendix A. At the end of an experiment, leaf area was measured by tracing around the tissue onto graph paper calibrated into square mm. Leaf area for both sides was then converted to square dm.

Intact plant resistance. As mentioned above, whole plant resistance can be calculated by measuring transpiration and

knowing the overall change in water potential from the nutrient solution to the air. In these experiments, the change in water potential was kept effectively constant (p.20), so that any changes in J would be attributable to changes in resistances and not to the driving force. Plants which had been in solution culture at least 4 weeks and had been in the growth chamber at least 4 days were used in all these experiments. Either 1 or 2 days before a plant was to be used, any leaves which could interfere with sealing and flowers or flower buds were removed with a sharp razor blade. Experimental runs took place in the same growth chamber that the plants had been growing in, and all experiments were initiated 4-7 h after the end of the regular light period. The chamber was kept dark except for a small shaded light to supply just enough light to prepare the experiment. A plant was carefully removed from its pot and quickly (5 s) reimmersed in distilled water in the bottom half of the root chamber section of the potometer. (See Appendix B for a plan of the root chamber.) With the plant on its side, electrician's tape was wrapped around the stem to ensure a proper fit in the pressure gland which seals the stem to the root chamber. Terostat VII (Terosan, Hamburg, Germany) was used to seal between and around the pressure gland inserts and the two halves of the root chamber. Once the terostat was properly placed, bolts were used to fasten the two halves of the chamber together and then, finally, the pressure seal was tightened. The chamber was then gently filled with distilled water (full volume, 3.6 l) and then pressure tested by closing off all valves except a column of water 40 cm high. When there was a

leak the chamber was disassembled, the terostat reset, and the chamber reassembled. The entire process described above was usually completed in less than 30 min.

When a successful seal was obtained, the chamber with the plant was suspended upside-down from steel rods so that the shoot was entirely enclosed in a plastic garbage container (height, 50 cm; diameter, 43 cm) and a polyethylene bag was then taped to the stem and the rim of the container in order to seal the shoot from outside air. The plant was suspended in this manner so that if wilting occurred and support lent by turgor was lost, the petioles or stem would not fall over and break. The air within the chamber was maintained at a constant low relative humidity (about 10%), and was mixed by three small fans blowing directly onto the foliage. Very dry air (RH = 4-5%) was obtained from the building compressed air supply and blown into the container. The rate of this air was normally quite high (13 l/min) in order to provide low relative humidity and a high rate of mixing within the container. Windspeed in the container was checked with a Hastings Air Meter (Model AB-27, Hastings-Raydist, Inc., Hampton, Virginia) and was found to be non-uniform but everywhere greater than 50 cm/s. Relative humidity was not the same for each experimental run, but was always 8 - 12%. During any one run, RH was normally maintained ±1%, but rarely as much as ± 2%. These variations would not be large enough to cause a measurable variation in the transpiration rate. Temperature was normally 24±1°C in the container.

When the plant was properly suspended and the container fans

and air supply were on, about one-half hour was allowed before water uptake (assumed equal to transpiration) was recorded to ensure that a steady state had been reached and that the system was working properly. Measurement of water uptake was done using the same apparatus and procedures described for the root resistance experiments (p. 21). In some experiments, the roots were irrigated in different salt solutions during a single experiment. This was accomplished by draining the existing solution and refilling with the next solution, using a funnel and plastic tubing connected to a fitting in the root chamber. The entire process took approximately 10 min, with the roots in air for about 6 - 7 min. of that time. A thin film of liquid always remained on the root surfaces, so appreciable root drying did not occur. Once filling was complete, the drain and refill tube was clamped off and the measuring system valve re-opened. Osmotic solutions were made as described earlier (p. 22).

In other experiments, the stem was cut with successively deeper cuts. A sharp razor blade was used and left in place after each cut. Cuts were made either on the hypocotyl or the first internode. Three or 4 cuts of increasing depth were made about 2 mm apart and the experiment terminated by complete cutting. Transpirational flux was measured for about 30 min after each cut. After the experiment the length of stem containing the cuts was cut out and observed under a dissecting microscope to determine the number of bundles severed with each cut.

RESULTS AND DISCUSSION

VESSEL RADII AND FREQUENCY DISTRIBUTION

Roots. Microscopic examination of root sections showed xylem arranged in a tetrarch pattern, with one very large metaxylem member at the center. This arrangement was consistent along the entire root. The very finest roots had only one group of 3 vessels which were centrally located. Staining showed that lignin had formed in vessel walls of all sections taken.

Table 2 presents data on the location of sections taken and the sizes of vessel members. Since members in the sections were circular, actual rather than Preston's (1938) modifications are given. The large centrally located metaxylem member was 4 - 5 times larger in radius than the other metaxylem members. The values given for the smaller metaxylem members are means of the 8 - 12 members present.

The relative role of each vessel in water transport (assuming all were water filled) can be calculated using Poiseuille's law (equ. 4, p. 7). Taking all the radii of a section to the fourth power, summing, and calculating the percentage contribution of each radius gives the percentage role of each vessel. For all the root sections which had a central metaxylem member, about 98% of the flow would be expected to occur through that member, with the rest divided among the 8-12 other smaller members.

Stems. The vascular system in the roots makes a definite transition into the stem vascular system as described by Hayward (1938, p. 600). In the mature stem there are typically 10 - 12 bundles, arranged in two rings, the outer of which has

Table 2. Locations, sizes of root, and sizes of vessels in the root system of a mature squash plant. "C" refers to the centrally located metaxylem member.

Location	Diameter of root (mm)	Average radius of vessel members (μm)
root tip	1.2	85 (C)
		19
mid-root	1.2	55 (C)
		10
root near the stem	1.5	120 (C)
		25
finest root tip	0.4	11

slightly smaller bundles.

Freehand sections taken midway along internodes showed that vessel members were usually ellipsoidal in shape. All measurements, therefore, were converted to the effective radius, as described on p. 18 . Since the deviation from circular was usually not great, the effective radius correction amounted to only about a 3 - 4% increase in calculated conductive capacity.

Three internodes were sampled for tracheary element sizes and frequency distribution. An average of 180 elements were counted in each section. Most of these had an effective radius of less than 75 μm (figure 1). As mentioned earlier, positive identification was not made of lignified cells of 5-10 μm effective radius, so the number of elements given in the 0-24 μm class may not be correct.

Figure 1 also shows the mean of the frequencies within size classes for the three internodes. At radii greater than 25 μm, there is an inverse relationship between the size of vessel members and their respective frequency. The largest effective radius observed was 127 μm. Although comparatively large for many plants, this is a value commonly encountered for ring porous woods and vines. Hayward (1938, p. 618) reports that squash vessels can have a radius of up to 250 μm.

Using Poiseuille's law, the relative role of each size class in water transport was calculated. Figure 1 shows the mean of the percent of total flux contributed by each size class for the three internodes. Vessels with effective radii from 50-124 μm were most important, with each effective radius class within that

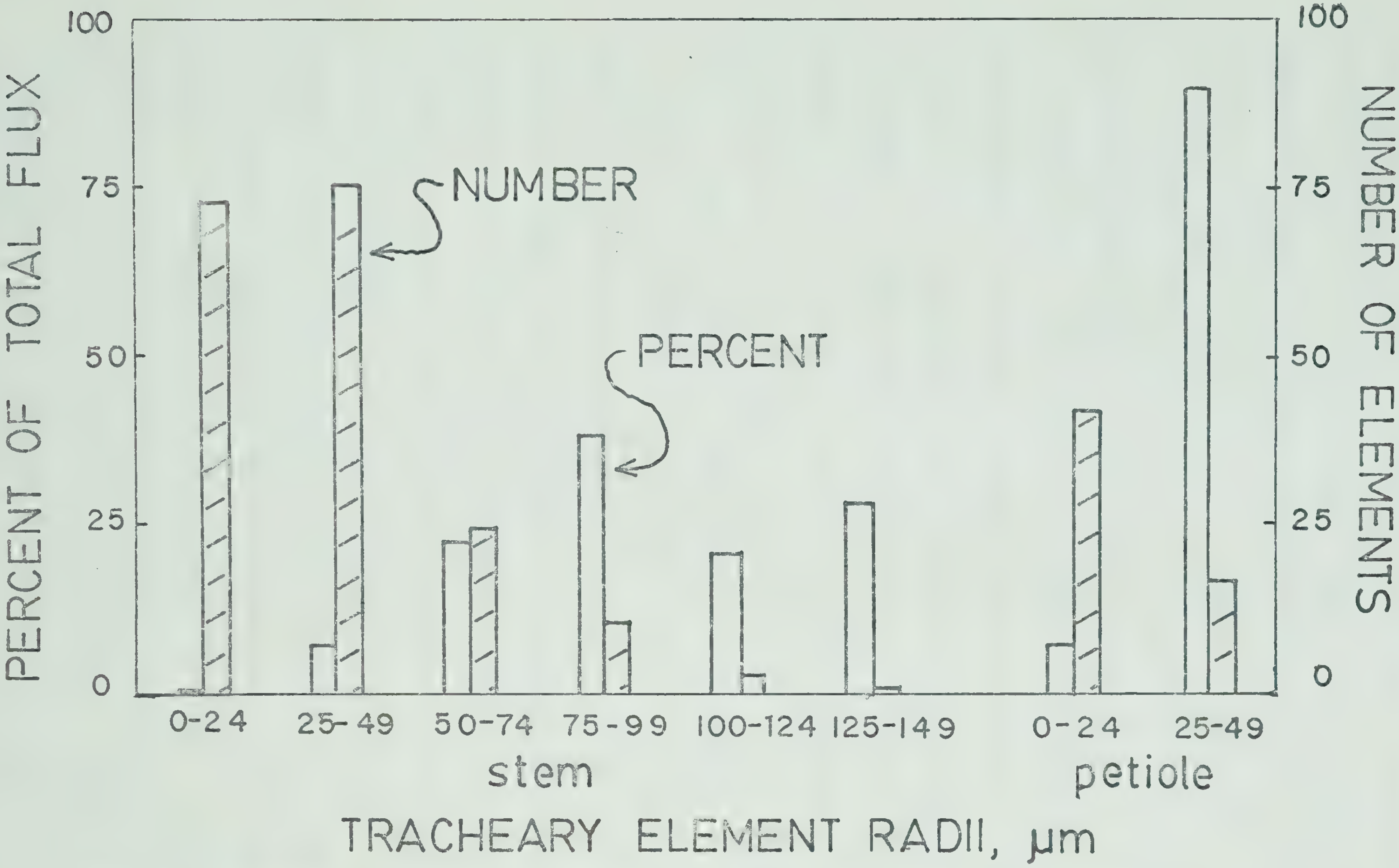

Figure 1. Number of tracheary elements in the stem and petiole of a squash plant and expected water flux through each size class of tracheary elements as calculated from Poiseuille's law. The data from stems are an average for 3 internodes, and the data for petioles an average of 11 petioles.

range contributing greater than 20%, and overall accounting for 90% of the flow. Interestingly, the single largest vessel (effective radius, 124 μm) accounted for nearly 30% of total water flux on its own. Loss of conductive capacity of this vessel would be associated with a redistribution of the relative role of each tracheary element and an increased resistance.

Overall, fig. 1 shows that the vascular system of the stem is biased towards the larger vessels as the most efficient conductors. In case cavitation occurs in the larger ones, many smaller conduits are present so conduction would still occur. Unfortunately, few frequency distributions of vessels radii for other species have been reported. Diamond (1966) found a distribution of vessel radii in tomato similar to those found in this study.

Petioles. Ten to thirteen vascular bundles are present in a circle surrounding the disintegrated pith in petioles of squash. Tracheary element size is much smaller in the petioles than in the stem, as fig. 1 shows. Distribution and percentage fluxes shown in fig. 1 are means of the 11 mature petioles which were sampled, and were calculated in the same manner as with the stem. In general, the same trend is found with petioles as with the stem, i.e., greater numbers of smaller conduits, with a few larger vessels responsible for most of the water transport. As might be expected petioles do not contain nearly as much vascular tissue as the stem and their resistivity is about 40 times that of the stem.

Leaf blade. Only limited data were collected from leaf blades. Identification of vessel members was difficult in the section taken, which included a secondary vein. The average radius of 8 vessel

members located in the vein was 45 μm. Most of the vessels or tracheids would be expected to be smaller than this. This radius is about the same as the largest found in the petiole, and this may indicate equal ease of cavitation occurring in either structure. Boyer (1971b), after dehydrating sunflower plants found evidence of cavitation occurring in stems (vessel radii: 30-66 μm), but not in petioles (vessel radii: 15-30 μm). This indicates an involvement of vessel size with ease of cavitation. Squash vessels in leaves, as well as roots, stems, and petioles, lie well within the 30-60 μm range in which Boyer found cavitation to be prevalent. These limited data suggest that cavitation of at least some vessels may occur anywhere in squash.

As mentioned above, it appears that squash is well adapted for efficient conduction as a result of its few large diameter vessels, yet it has considerable back-up with smaller and safer vessels and tracheids in case of extensive cavitation. These smaller elements also perform the additional function of providing support. The smaller the diameter of a tracheary element, the more axial strength it imparts. Thus xylem morphology cannot be considered to be only a result of selection in favor of an efficient and reliable transport system.

VESSEL HYDRAULIC CONDUCTIVITY

Hydraulic conductivity of 5 vascular bundles dissected from hypocotyls was experimentally determined and then compared to the theoretical hydraulic conductivity expected from Poiseuille's law (see p. 7). When expressed as a percentage of the theoretical value, hydraulic conductivity of these sections was 45, 59, 64, 74

and 74% ($\bar{x}$ = 63, s = 12). Zimmermann (1971, p. 199) showed other data which indicated some vines, presumably similar in vessel morphology to squash, had a conductivity equal to that given by Poiseuille's law. However, most species have hydraulic efficiencies of less than half of the Poiseuille value. The relatively low average value of 63% obtained with squash (in comparison to other vines) probably results from technical problems as well as inherent factors in squash xylem. For instance, it was difficult to accurately measure tracheary element sizes for even a 1.5 cm long piece of bundle. In one case, hydraulic conductivity of one sample deviated up to 23% from the mean of the four samples which were made. Next, it may be possible that some air blockage may have occurred and remained, even though cutting was done under degassed and distilled water and the slight hydrostatic pressure (0.005 MPa) would have helped to force air out.

Zimmermann (1971 p. 196) suggested that limited vessel length is the most important factor in decreasing xylem conductivity. Although vessel length was not measured in this study, the number of vessel endings seen in freehand sections suggested that vessels may be rather short. Remnant end walls of vessels members have been suggested by many investigators to present an additional flow resistance, but Hayward (1938, p. 29) found end walls almost nonexistant in squash at maturity. Hayward did find (p. 613) that tyloses were common in older vessels of squash. Freehand sections did occasionally show blockages in vessels, which appeared to be protoplasm. Tyloses form in response to air in the vascular system, suggesting again cavitation and resulting embolism

(Zimmermann 1971, p. 213).

The Hagen-Poiseuille equation (equ. 4) may be re-arranged to calculate an approximate value for the hydrostatic pressure through the vascular system of squash. Doing this, and adding the factor to allow for deviation from ideality, gives:

$$\Delta p = \frac{8\ J\ \eta\ \ell}{(0.63)\ \pi\ \Sigma r^4} \quad . \tag{8}$$

It is assumed that the factor accounting for deviation from ideality (0.63) is the same for all of the xylem in the vascular system (roots, leaves, and stem other than hypocotyl).

Using the values obtained as described above for Σr^4, (p.17), J under selected conditions (from Appendix A), $\eta = 0.095$ poise (22°C), and reasonable values for ℓ, allows calculation of a rough estimate of the hydrostatic pressure drop in the xylem of a squash plant (Table 3). To simplify calculations, pressure drops are calculated for only the stem and petiole. The vascular system in the leaf blade and roots is quite complicated, and cannot be considered without further study of morphology. However, since the vessel radii in both the leaf blade and roots are about the same size as in the petiole, pressure drops are probably about the same as in the stem and petiole. When the stomata are open in the light, Δp is larger in both petioles and the stem. With reduced flow rates due to stomatal closure in the dark Δp is much smaller. The drop expected (although underestimated here) is very small in comparison to the measured drop in water potential from substrate to leaf, Appendix A shows this to average about 0.63 MPa when the

Table 3. Expected drop in hydrostatic pressure (Δp) calculated on the basis of Poiseuille's law. Reasonable values of length (ℓ) of a stem and petiole are given, along with rate of water flux (J) obtained from Appendix A. Environmental conditions assume uniformly low ambient relative humidity. In the light, stomata are open, and in the dark, they are closed.

Plant part	ℓ (cm)	J (μl/min)	Conditions	Calculated Δp (MPa)
Stem	50	500	light	0.01
		20	dark	0.0004
Petiole	25	63	light	0.01
		2.5	dark	0.0005

roots are in water and the shoot is in low relative humidity and light. Most of the drop in water potential is apparently due to resistances other than the xylem. Since Δp is so small along the xylem, any changes in the resistance to water flux in the xylem are difficult to measure because Δp could change slightly and never be detectable.

ROOT RESISTANCE

In the first set of root resistance experiments water flux due to root pressure and hydrostatic pressure applied to the roots was determined. Root pressure was first observed for 90 min (fig. 2) to ensure that root resistance was not varying markedly. Although water flux due to root pressure decreased by about 70% in 90 min in one case (fig. 2A), this is considered unusual. More typically, a constant rate was observed (fig. 2B). Water flux was constant and of about the same magnitude whether the roots were in distilled water or Hoagland's solution.

With the application of hydrostatic pressure, an elevated rate of water flux was initially observed, followed by a decrease with time to a steady rate (fig. 2). This type of response was quite repeatable. Of the experiments in the literature in which water has been pushed out through root systems or pulled by vacuum at a cut stem, none have shown the response observed here with squash. Kuiper and Kuiper (1974) have even shown an opposite response: a rise to an asymtotic value. Based upon Weatherly's (1963) analysis of water fluxes in response to abrupt changes, water may be transferred from storage areas within the plant (e.g.: cell walls) upon the pressure increase. Such a transference

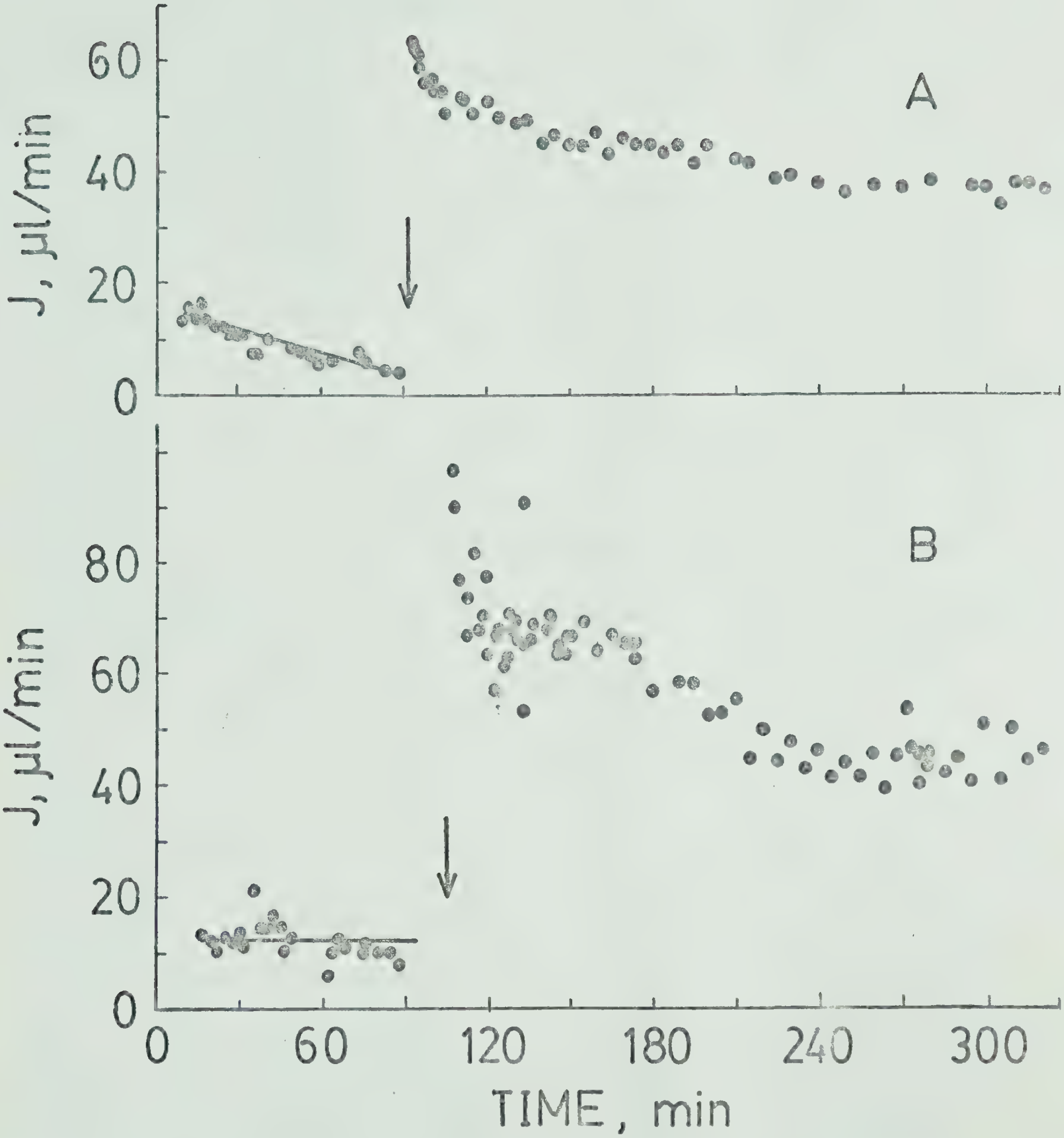

Figure 2. Rate of water flux through root systems of young squash plants. Initial flux rates are due to root pressure. The arrow signifies application of 0.5 MPa hydrostatic pressure to the root system.

would be associated with a lower resistance than root resistance, causing a higher than expected rate. The steady rate observed after several minutes probably reflects true water uptake. In any case, the data serve adequately as a control for testing the effect of dehydration on root resistance.

The second set of experiments was designed to investigate both the effect of dehydration on root resistance and to aid in the selection of an effective osmotic agent. The general procedure was to first establish a rate of water flux due to a certain hydrostatic pressure, then substitute the substrate solution of distilled water with an osmotic solution which would exactly cancel the hydrostatic pressure, and then finally, apply a hydrostatic pressure exactly twice that of the original. If the rate remained the same, no change in root resistance could be attributed to dehydration. If it did, root resistance did change with dehydration. Figure 3 shows the result of an experiment conducted wherein precautions were taken to quantify each phase of the treatment very carefully. Mannitol and NaCl, both in the presence of Ca^{++}, where also compared. A relatively smaller root system was used for this experiment. This resulted in lower rates than found in other experiments in this section. The abrupt initial increase and following decrease in J usually found after the increase in hydrostatic pressure was not consistently observed during this experiment. The reason for this is unknown. Figure 3 shows that a rate was established for a hydrostatic pressure of 0.5 MPa when the substrate of distilled water was replaced with mannitol solution having an osmotic potential of -0.5 MPa.

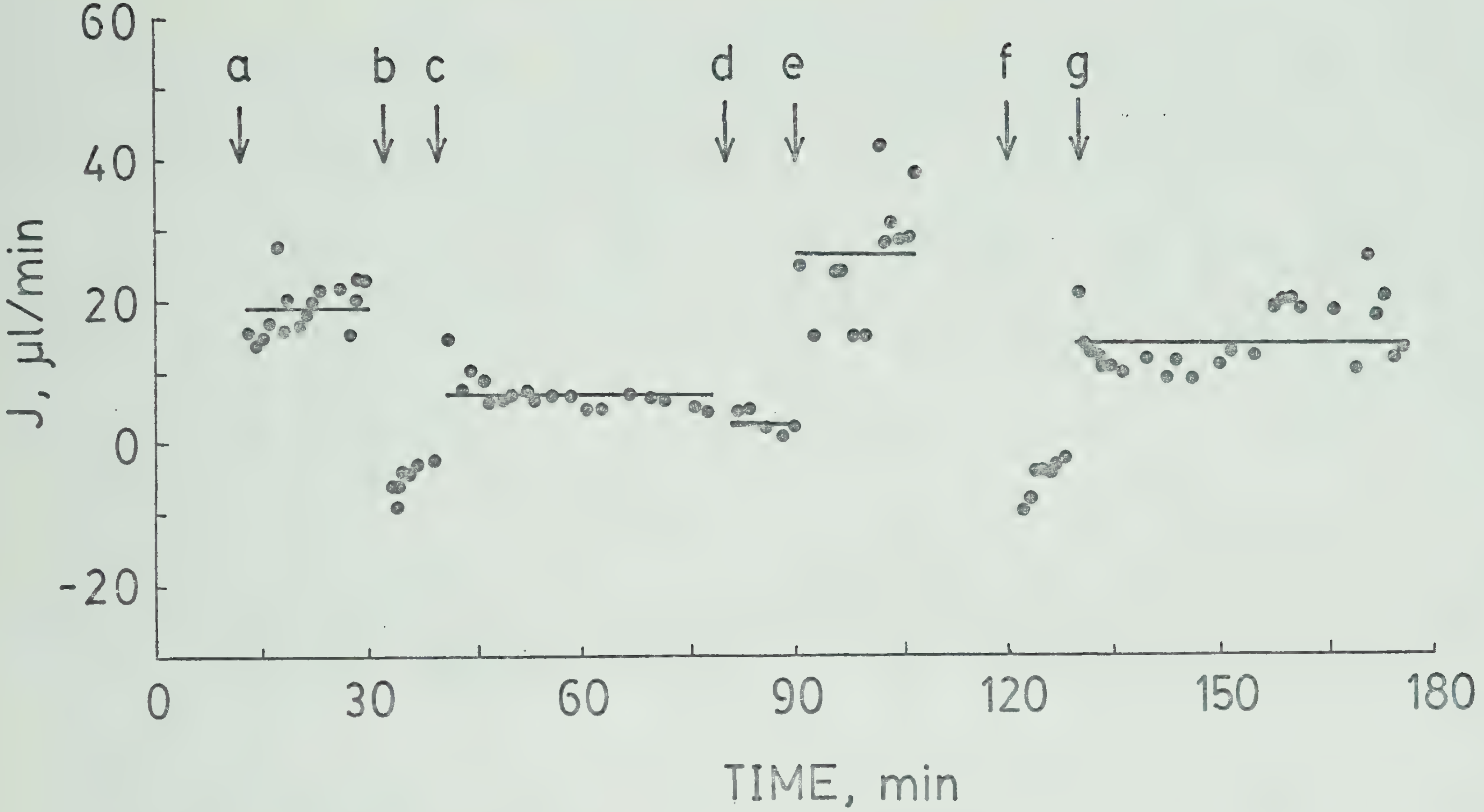

Figure 3. Fluxes of water into and out of a root system of a young squash plant resulting from pressure and osmotic treatments. Negative fluxes occur when water moves out of the root system into the substrate solution. The arrows indicate the following: a) substrate: distil [illegible] (osmotic potential (π) = 0), hydrostatic pressure (p) = 0.5 MPa; b) substrate: mannitol (π = -0.5 MPa), p = 0; c) substrate; mannitol (π = -0.5 MPa), p = 1.0 MPa; d) substrate: distilled water (π = 0), p = 0; e) substrate: distilled water (π = 0), p = 0.5 MPa; f) substrate: NaCl (π = -0.5 MPa), p = 0; g) substrate: NaCl (π = -0.5 MPa), p = 1.0 MPa.

Before hydrostatic pressure was applied, J actually reversed directions and water moved from the root to the mannitol solution. After a few moments, this negative flux decreased asymtotically to a small value. Upon an increase of hydrostatic pressure to 1.0 MPa, a positive flux was once again observed, but it was only about one-half of that formerly observed for the same overall difference. When the hydrostatic pressure was removed and the roots reimmersed in distilled water, root pressure was observed indicating viability of the root system. Before treatment with NaCl, a rate with the roots in water only and with hydrostatic pressure was once again obtained. This time, J was slightly but significantly (at the 99% level) higher than before under the same conditions. NaCl of osmotic potential -0.5 MPa was then added, and a similar negative flux was observed as with mannitol. With the increase of hydrostatic pressure to 1.0 MPa, a positive flux was observed again. As found for mannitol, J was only approximately one-half of the rate expected when both resistance and water potential difference were constant.

Repetition of the above experiment was attempted ten times, but only one trial could be relied upon. In the others, air often suddenly started coming out of the cut stem in the middle of the experiment, as if the root system was suddenly damaged. In other instances, consistent readings of J could not be obtained. The result of the one successful trial is shown in fig. 4. This experiment was a simplification of the one discussed above. NaCl was the only osmotic agent used, and only positive fluxes were quantified. The flux of water due to root pressure and hydrostatic

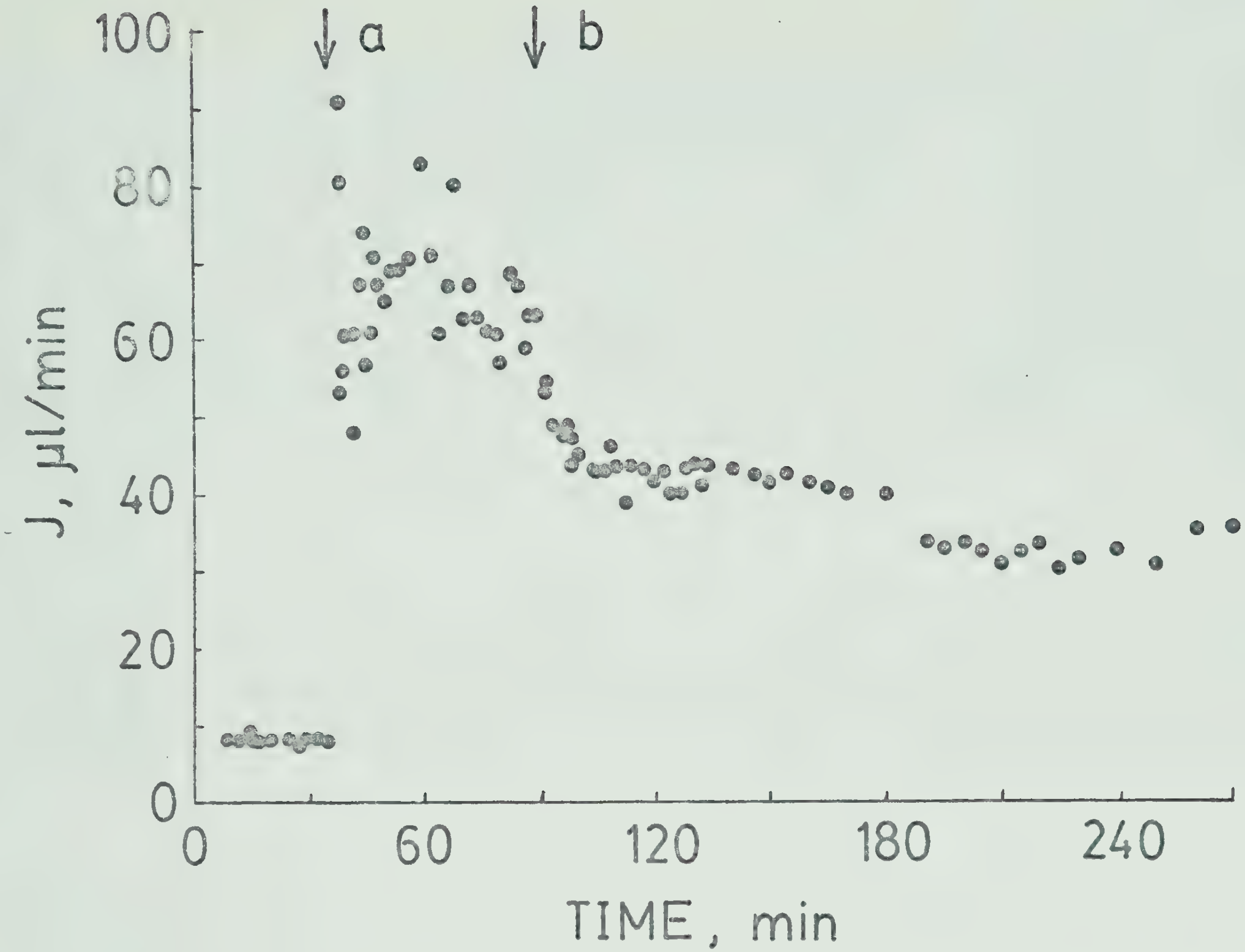

Figure 4. Flux of water through a root system of a young squash plant resulting from pressure and osmotic treatments. Initial rate is due to root pressure. The arrows indicate the following: a) substrate: distilled water (osmotic potential (π) = 0), hydrostatic pressure (p) = 0.2 MPa; b) substrate: NaCl, (π = -0.2 MPa), p = 0.4 MPa.

pressure (0.2 MPa) was similar to that given by the controls (fig. 2). With the addition of the osmoticum (-0.2 MPa) and the increase of hydrostatic pressure to 0.4 MPa, J decreased quickly and to a greater extent than predicted by the controls. Although the change is slight, there does appear to be a definite increase in root resistance associated with osmotic treatment in this experiment, as well as the preceeding one (Fig. 3). Furthermore, in the preceeding experiment a stronger solution was used (-0.5 MPa vs. -0.2 MPa in fig. 4) and a greater decrease in the rate was observed with osmotic treatment. This possibly indicates a dependence of the resistance change upon degree of dehydration. The treatment apparently does not damage the root system since root pressure was observed when the roots were returned to water after an osmotic treatment (fig. 3). More experimentation and possibly refinement of technique will be necessary to assess this problem more carefully. It is not a critical point in relation to this work, since even large changes in root resistance probably have little effect on water flux in intact plants when the stomata are closed, as future calculations will suggest (p. 50).

Exactly how dehydration affects root permeability is unclear, but at least one mechanism has been envisioned by investigators. Ordin and Gairon (1961) found that diffusion of tritiated water into young Vicia faba L. root systems was increased by water stress, while water stress had no effect on diffusion of the same isotope into maize roots. It appears that such an effect is variable and species dependent. They attributed such resistance changes to loss of turgor and resulting realignment of macromolecules, particularly proteins. Conclusive evidence on this

point is lacking.

LEAF RESISTANCE

As figure 5 shows, detached leaf sections in the dark undergo an immediate sharp decrease in the rate of water loss and then a more steady decrease in rate of loss after about 1 hour. Leaf water potential changed slowly during the experiments, and never decreased below -2.4 MPa. Therefore, intercellular spaces remained nearly saturated with water vapor and the driving force for evaporation remained constant. Leaf resistance (R_1) was calculated by the formula given by Slavik (1974, p. 337). Boundary layer resistance was assumed constant and negligible. After 2 min R_1 was 17.5 and 16.8 s/cm for trials 1 and 2 respectively, and after 450 min, R_1 was 103 and 132 s/cm, respectively.

The very large rates of transpiration which occurred initially in these experiments are quite likely due in part to freely occurring evaporation from the cells damaged when cutting the leaf section out. It may also be that stomata are not fully closed in the dark. With detachment of the leaf, turgor is lost and full closure occurs. Holmgren et al. (1965) suggested that stomata were responsible for a portion of "cuticular transpiration" of leaves kept in the dark, and Williams and Amer (1957) found "cuticular transpiration" higher from leaf surfaces with stomata than those without. Mayo (personal communication) has found a species of orchid which does not normally close its guard cells fully in the dark. This is probably due to epidermal and guard cell turgor relationships. If squash stomata are normally slightly open in the dark, the higher rates observed initially may

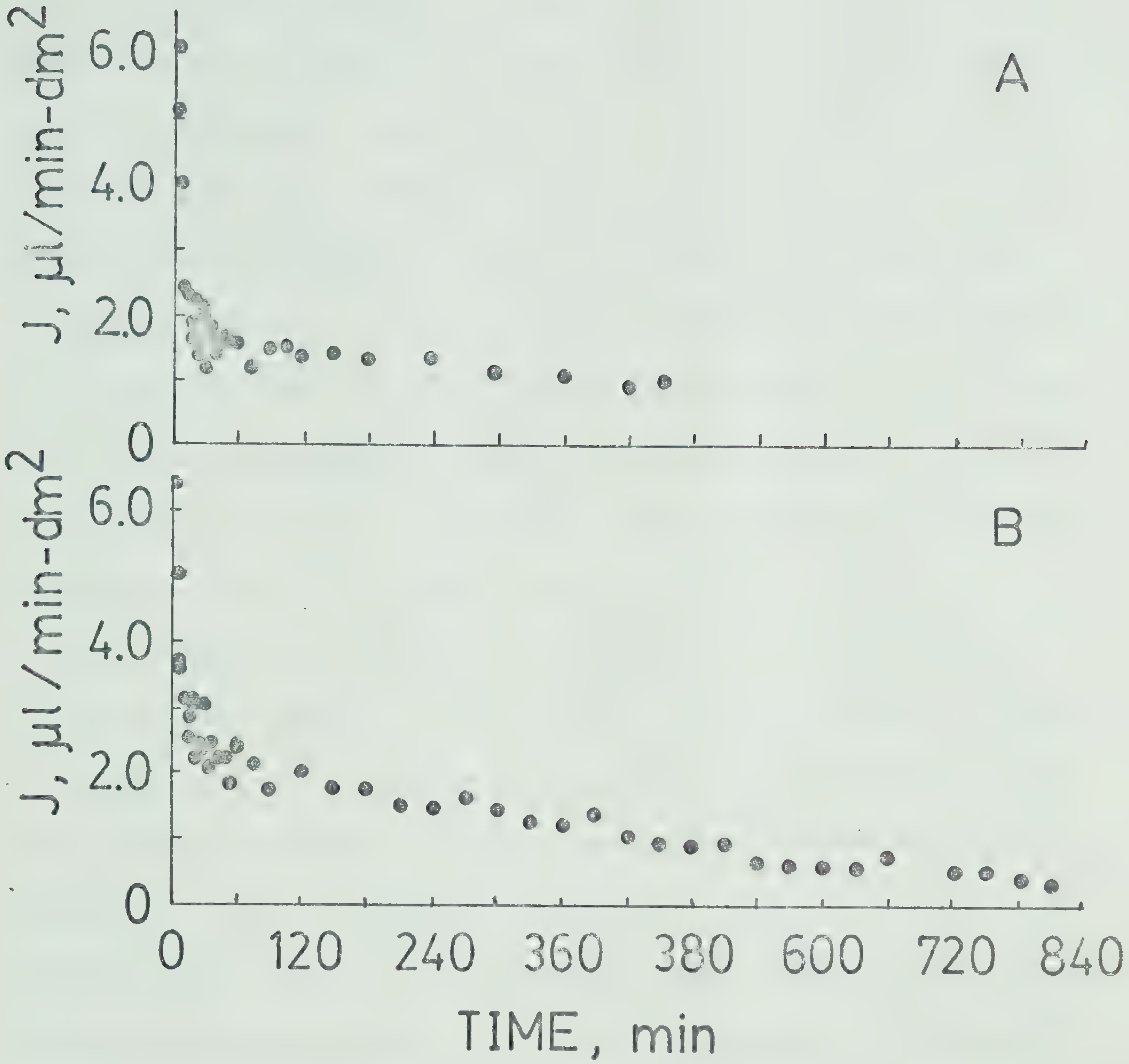

Figure 5. Rate of water flux per unit surface area from 2 detached sections of squash leaves in the dark. A: Relative Humidity(RH) = 15 ± 1%, air temperature (T) = 23.0 ± 1.0° C, leaf area = 0.62 dm^2, initial leaf water potential (ψ) = -0.6 MPa, ψ at 810 min = -2.2 MPa. B: RH = 9 ± 1%, T = 22.0 ± 0.5° C, leaf area = 0.46 dm^2, initial ψ = -0.6 MPa, ψ at 450 min = -2.4 MPa.

reflect a small amount of stomatal transpiration as well as evaporation from the cut edges. In attempts to determine if this was so, similar experiments using whole leaves were carried out. Unfortunately transient increases in water loss were found with whole leaves, which confused results. This transient effect, the Ivanov effect (Ivanov 1928), has been commonly observed. It probably results from the release of tension in the xylem from cutting and consequent changes in guard cell turgor. With detached leaf sections, veins were excluded from the section, so not enough water would be available to produce the Ivanov effect. If the Ivanov effect did occur with detached leaf sections, it may simply have been hidden by the high initial rates.

The final steady decrease in transpiration rate, indicative of increasing resistance, may be due to several factors. Firstly, cuticular resistance may change as drying occurs (Schönherr 1976; Dubé 1975) or pathways of evaporation within the leaf may change. Secondly, since the supply of water to the leaf has been removed, water must be transferred from other sources such as protoplasts, and the characteristics of flux from these sources are likely to be much different than from the xylem. Leaf resistance may or may not change greatly with leaf water potential in the dark in intact plants. If it does it may serve to effectively mask any changes in xylem resistance.

INTACT PLANT RESISTANCE

Effect of mechanical interruption. Before any treatments were attempted, control plants were tested for any oscillations or large variations in J in the dark. As fig. 6 shows, a moderate amount of

variation may be expected in any experiment. However, no regular oscillations were apparent during the 4 h runs. In these experiments, as in the following ones, there was not always a consistent value obtained for J when expressed on a leaf area basis. Therefore, since it is changes in fluxes of individual plants which are of interest, J alone is plotted in all the following figures.

Figures 7 and 8 show results of severing and blocking off vascular bundles in a stepwise manner. There is generally no effect on the rate of water uptake, even when very large portions of the stem are severed. In two cases small (10%) but significant (at the 99% level) decreases in J were observed after cutting most of the bundles, indicating a possible increase in xylem resistance. In one of these cases, shown in fig. 7B, it appears that the cutting has been imposed upon variations in J which would have occurred in spite of the cutting, thereby confusing the results. The pattern that J followed in this case was occasionally observed in other experiments as well (p. 52).

The maintenance of a constant rate of water uptake with a portion of the xylem conduits broken would be expected only if Δp were increased simultaneously, in order to overcome the increased resistance. The magnitude of this would not need to be great. For example, using the Hagen-Poiseuille equation (equ. 4, p. 7) if the xylem resistance term, Σr^4 is decreased by 90%, a 10-fold increase in Δp would be necessary in order to maintain J. Using the data given in Table 2, in the dark, Δp along 50 cm of stem and and 25 cm of petiole would decrease from -0.0009 to -0.009 MPa; in the light, the Δp in an identical system would decrease from

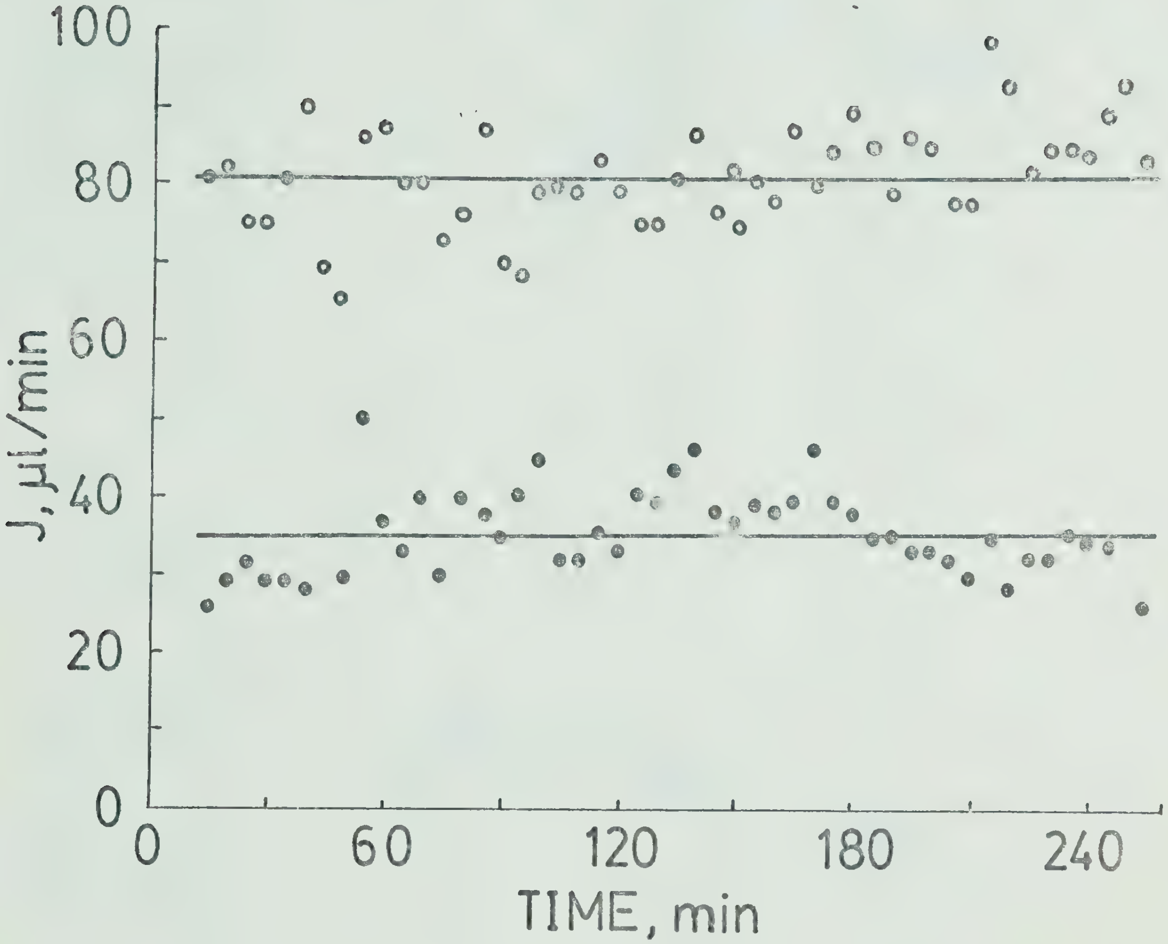

Figure 6. Rate of water flux through two intact squash plants in the dark over 4 hours. The roots were immersed in distilled water.

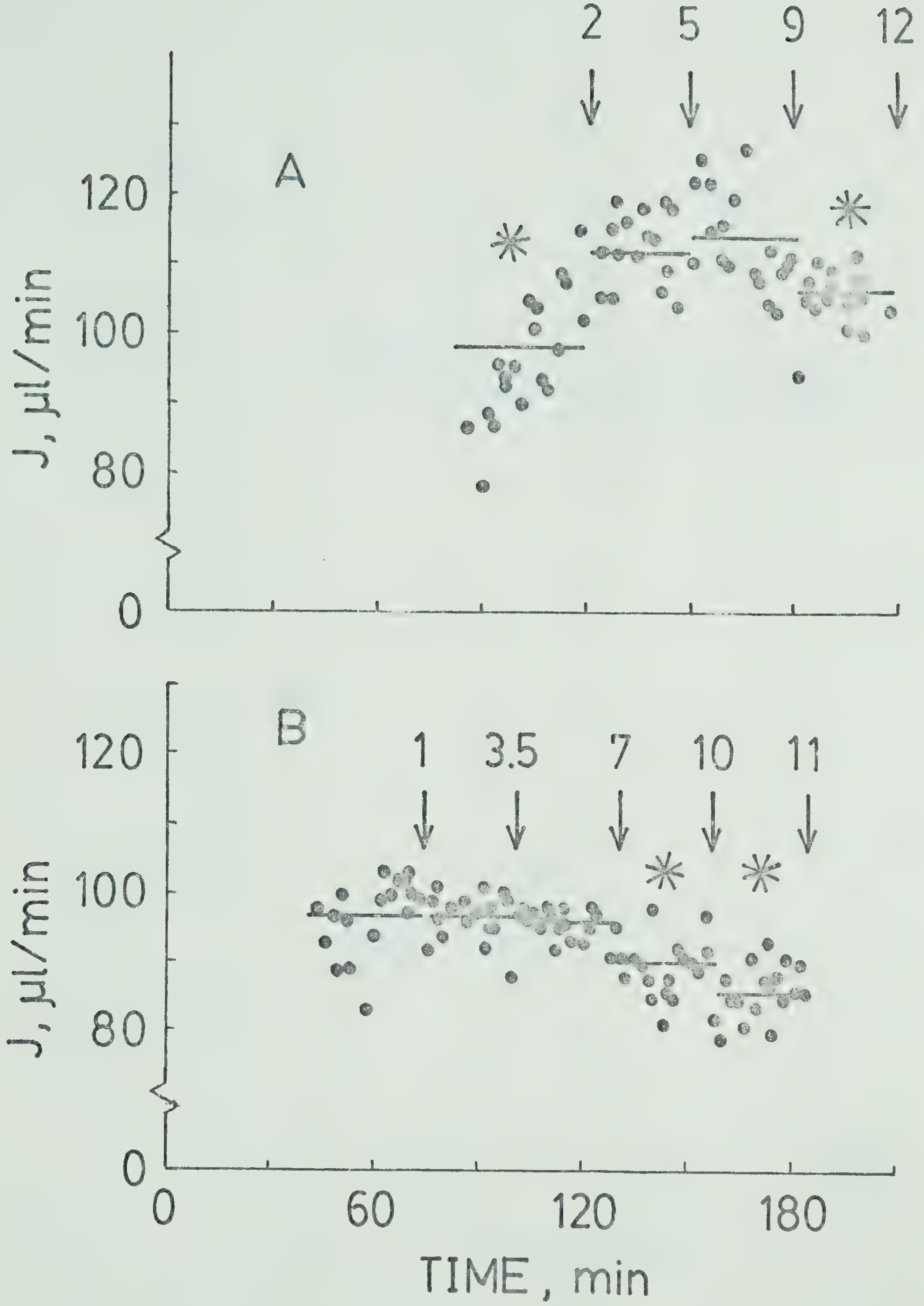

Figure 7. Effect of stepwise severing of stem vascular bundles on water flux in two squash plants in the dark. The roots were in distilled water. Initial rates are before any cutting. The arrows indicate a cutting. The accumulated number of vascular bundles cut is given. The plant used in A had a total of 12 bundles and the plant used in B had 11. Results which differed significantly (99% level) from the others are indicated with a *.

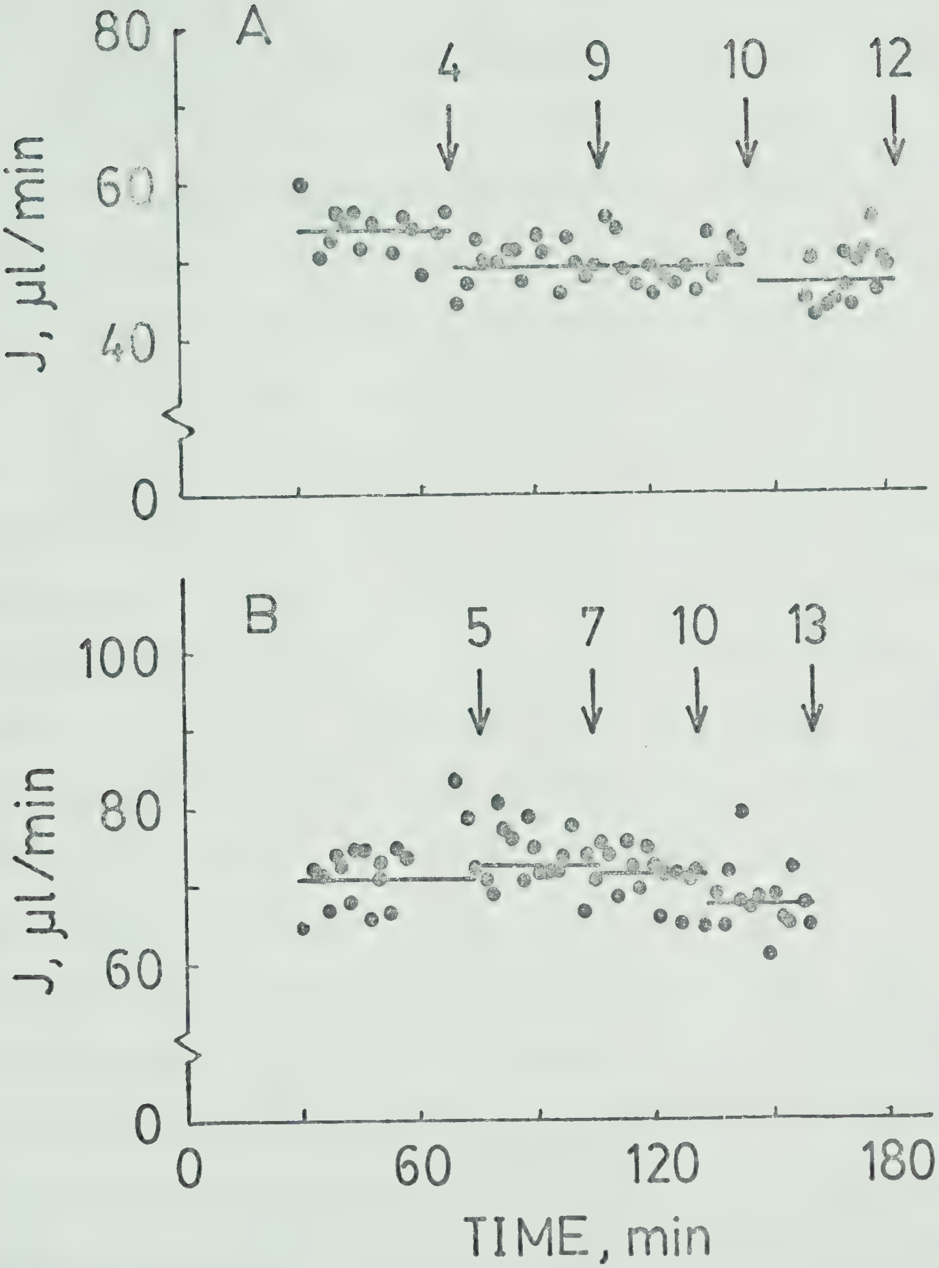

Figure 8. Effect of stepwise severing of stem vascular bundles on water flux in two squash plants in the dark. The roots were in distilled water. Initial rates are before any cutting. The arrows indicate a cutting. The The accumulated number of vascular bundles cut is given. The plant used in A had a total of 12 bundles and the plant used in B had 13. No significant statistical difference (95% level) was found between the treatments.

-0.02 to -0.2 MPa. These are very small changes and the best techniques used to measure ψ have a resolution of only about 0.1 MPa. Using the most sensitive method of measuring leaf water status, beta-gauging, Mackay and Weatherly (1973) were unable to detect any lowering of leaf water potential even when up to 90% of the stems of Acer or Gossypium were severed and blocked.

Consideration of the vascular system of squash suggests that severing the majority of vessels at one point along the stem would have little effect on J, even if Δp were constant. Firstly, since the embolism would be restricted to those vessels which are broken by the razor and vessel length is limited, relatively few vessels may be injured by the cut. Secondly, since each bundle on the average anastomoses at every second node with other bundles (Hayward 1938, p. 614), redistribution of water could occur so that downstream from the cut all vessels would still have a source of water. Therefore, little conductive capacity would be lost when a single cut was made. Unfortunately, it is not experimentally feasible to make cuts at every internode simply because some entire leaves may be cut off from their supplying vascular bundles.

Caution must be exercised in interpreting these experiments because measurement sensitivity to changes in xylem resistance is not great. Since the xylem (or the root) offers such a small resistance in comparison to the rest of the pathway when the stomata are closed, changes in its characteristics may have little affect on the overall rate. Although the relative magnitudes of the pathway resistances are not known for squash, this point can be illustrated by assuming the relative resistances in squash are

similar to those described by Boyer (1969) for sunflower. For sunflower with open stomata, he found that the ratio of the resistance of the root, stem and leaf was 2:1:1. Using this ratio for squash with reasonable values for J with open stomata (15 $\mu l/dm^2$-min) and closed stomata (0.50 $\mu l/dm^2$-min) from Appendix A, gives a ratio of 2:1:15/0.50 or 2:1:30 for squash with closed stomata. In the case of closed stomata, xylem resistance accounts for (1/33) x 100 or about 3% of the total resistance. Root resistance also makes up a small portion of the total, only about 6%. Practically, either of these resistances could change markedly, and yet the measurement system used in these experiments would never show an appreciable change in J.

Although these experiments show a possible increase in xylem resistance as a result of mechanical interruption, any such change is very small. It is not known how much of the vascular system is actually affected by a single cut, but it appears that only a small portion is injured. Furthermore, since flow is limited mainly by the cuticle, there is very little sensitivity for measuring changes in the resistance of the xylem and only large changes are detectable.

Effect of decreasing substrate solution osmotic potential. Cavitation might possibly be induced by high tensions placed upon the xylem. Since such cavitations may commonly occur anywhere in the plant a change in xylem resistance may be observable, whereas the cutting experiments showed none. The following experiments were designed to test this on intact plants in the dark by treating the roots with osmotic solutions. In order to decrease

the substrate solution osmotic potential, an appropriate way in which to add osmotica to the root chamber was sought. Initially, in order to avoid exposure of the roots to air, it was thought that by adding a very concentrated volume of salt solution to the chamber, and mixing it vigorously within the chamber with a peristaltic pump, the substrate solution osmotic potential could be satisfactorily lowered. However, as fig 9 shows, recirculation of water alone within the root chamber brought about a dramatic decrease in J. This phenomenon was also found to occur when the root chamber was vigorously aerated.

Next, complete drainage and refilling of the root chamber was tested as a way to decrease substrate osmotic potential. With the root chamber containing distilled water, one-half of the total volume was drained, and then immediately refilled with the same water. An abrupt drop in J was apparent (fig. 10). Next, in the same experiment, slightly more than one-half of the water was drained and immediately refilled, resulting in a further stepwise decrease in J (fig. 10). As fig. 10 also shows, the initial values of J were not steady, as earlier experiments (fig. 2) had shown. However, after 90 min, a steady rate was observed. The initial oscillation was quite probably due to an adjustment to the slightly drier atmosphere of the shoot chamber, a process which simply took longer in this experiment than in others.

Even though the drainage treatment seems to be a more severe treatment due to the fact that the roots are in air 6 - 7 min, and since both methods affect J similarly, the drainage treatment was chosen due to its ease of application. Several more experiments

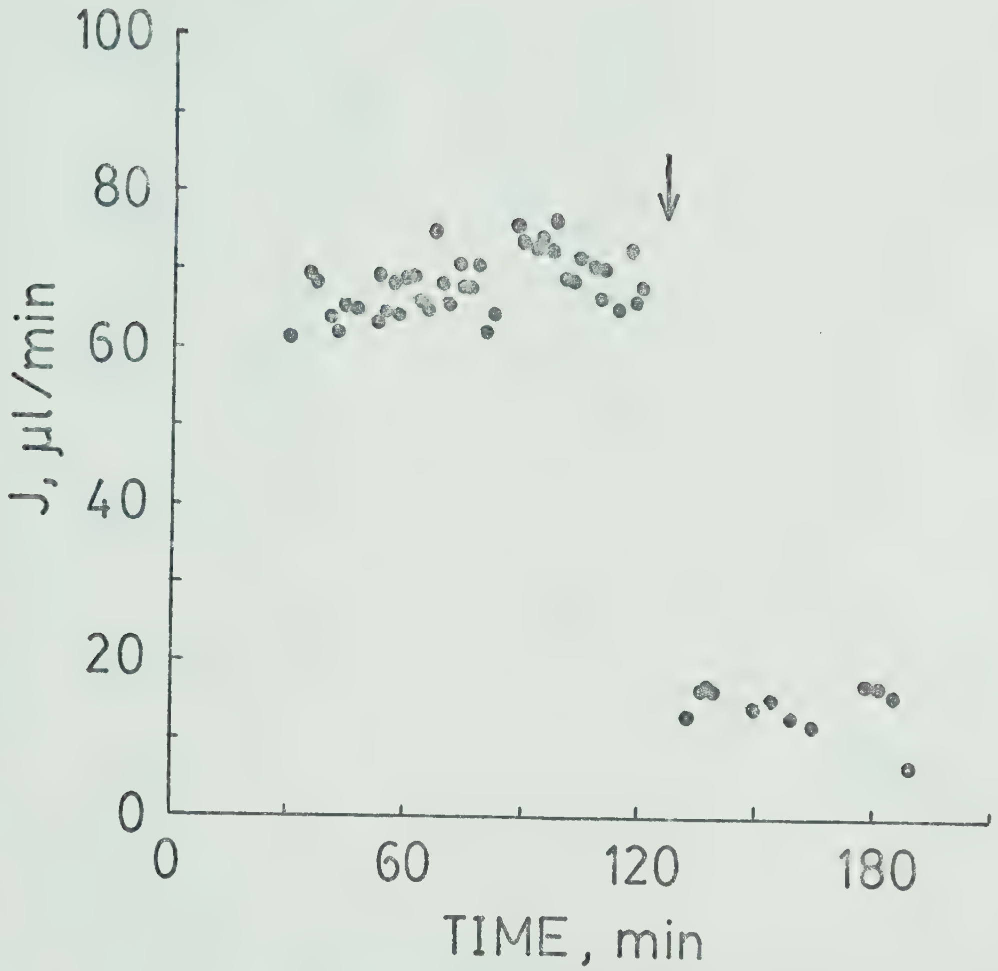

Figure 9. Effect of peristaltic mixing of the substrate solution upon water flux in a squash plant in the dark. The substrate was distilled water. Mixing began at the point indicated by the arrow.

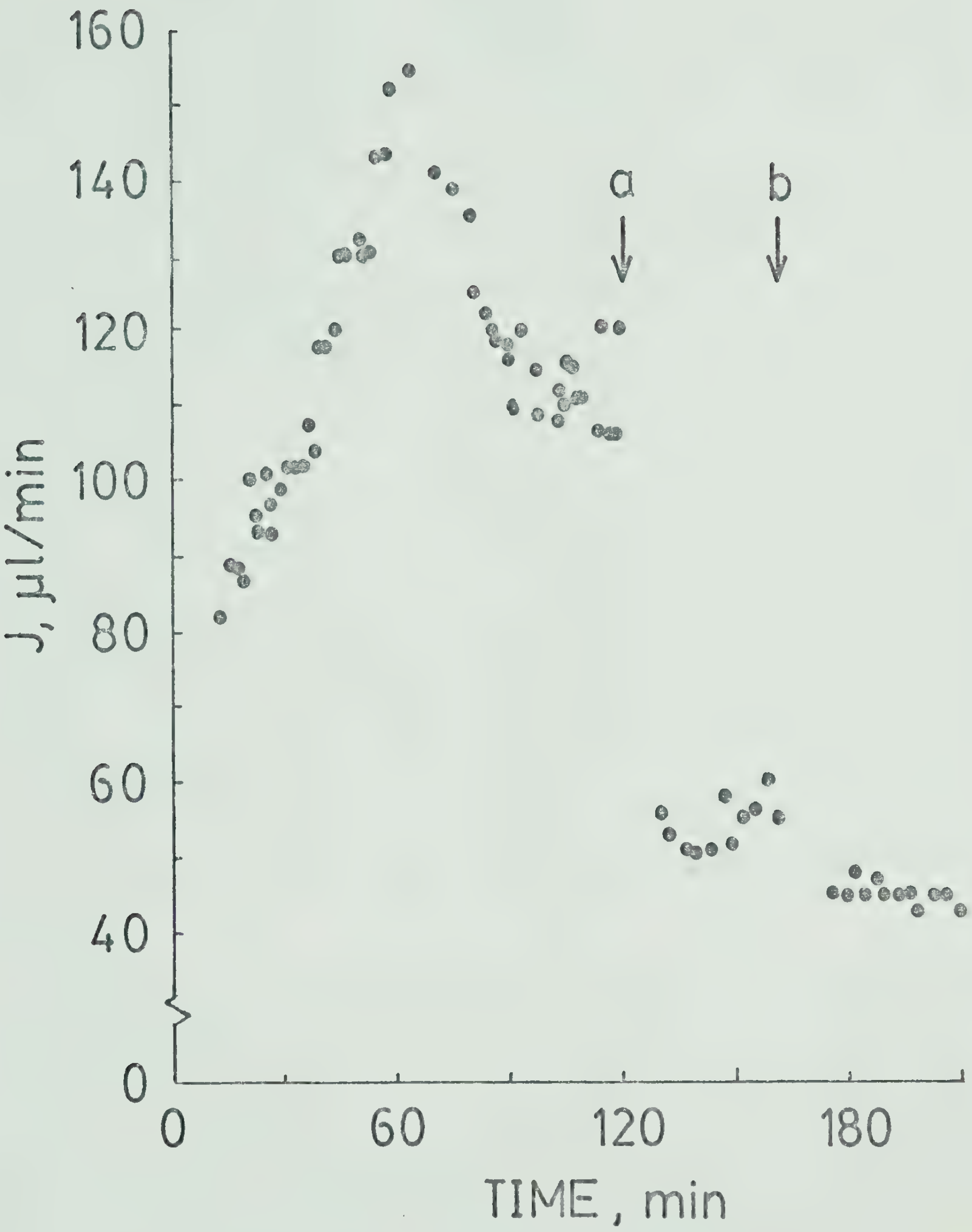

Figure 10. Effect of stepwise drainage of the substrate solution upon the rate of water flux in a squash plant in the dark. The substrate was distilled water. Drainage of about 50% of the substrate solution was commenced at a. Drainage and refilling of about 60% of the substrate solution was commenced at b.

were done, to serve as controls, and to see if additional drainages had a further effect on J. In these experiments, initial rates were observed in water, then the water drained and the chamber refilled with a salt solution of -0.45 MPa. Salt solutions were used to increase value as controls. Such a weak solution was not sufficient to cause wilting, but it would be expected to have an effect on leaf water potential. The root chamber was drained and refilled with the same solution 5 or 6 times in a single experiment, and as figures 11, 12 and 13 show, J stayed approximately the same throughout. A slight increase in one experiment and a slight decrease in another were noted. The decrease in J noted with the first refilling was approximately the same in these experiments as expected with water (fig. 10), so no effect of the -0.45 MPa salt solution was apparent. Since the additional drainage had little or no further effect on J, this method is clearly appropriate for inducing stress by decreasing the substrate solution osmotic potential.

Figures 14 and 15 show the results of treating the roots with salt solutions of -0.45, -1.0, -2.0, -3.0, and -4.0 MPa osmotic potential. The rate of water flux remained approximately constant throughout each treatment (lasting 30 min each). Minor variations beyond those found in the controls were apparent in one case (fig. 15B). All of the plants wilted during the experiment, and the wilting eventually progressed to the point where shrinkage of the stem caused an irrepairable leak at the pressure gland inserts. Leaf water potential changed very slowly during the experiment, reaching only as low as about -0.12 MPa, a depression of only

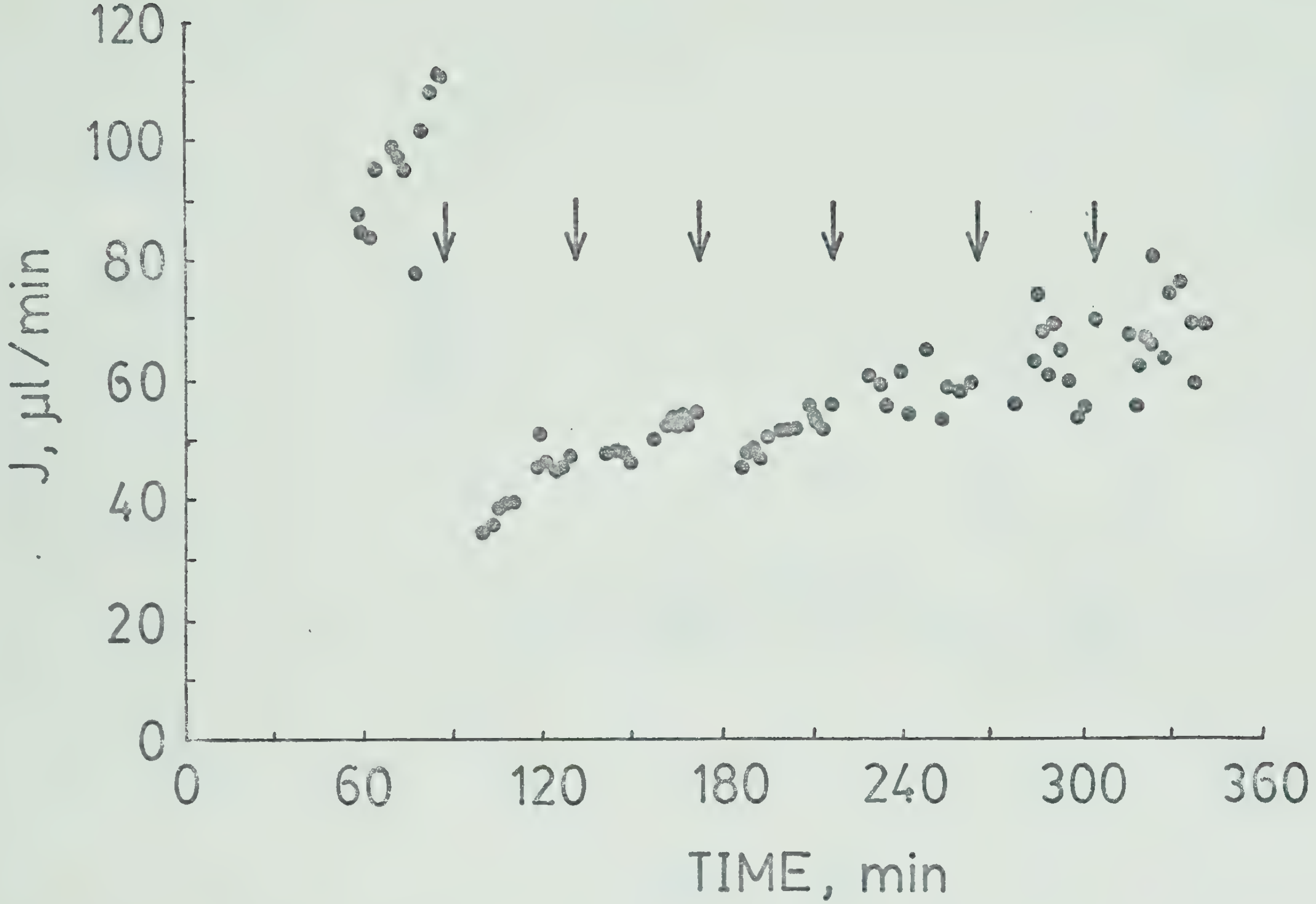

Figure 11. The influence of sucessive entire drainages and refills of the substrate solution upon water flux in a squash plant in the dark. The substrate solution was NaCl of -0.45 MPa osmotic potential. Each arrow indicates commencement of a drainage and refill.

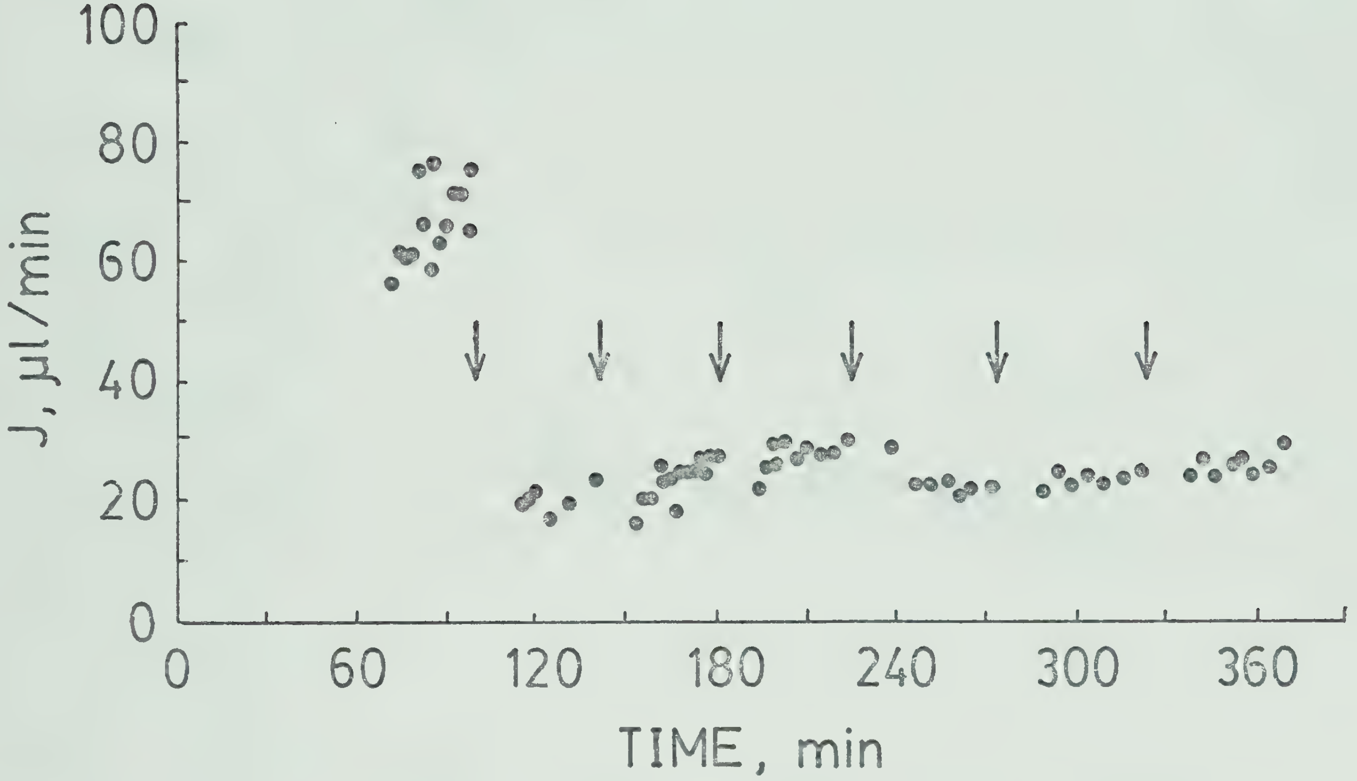

Figure 12. The influence of sucessive entire drainages and refills of the substrate solution upon water flux in a squash plant in the dark. The substrate solution was NaCl of -0.45 MPa osmotic potential. Each arrow indicates commencement of a drainage and refill.

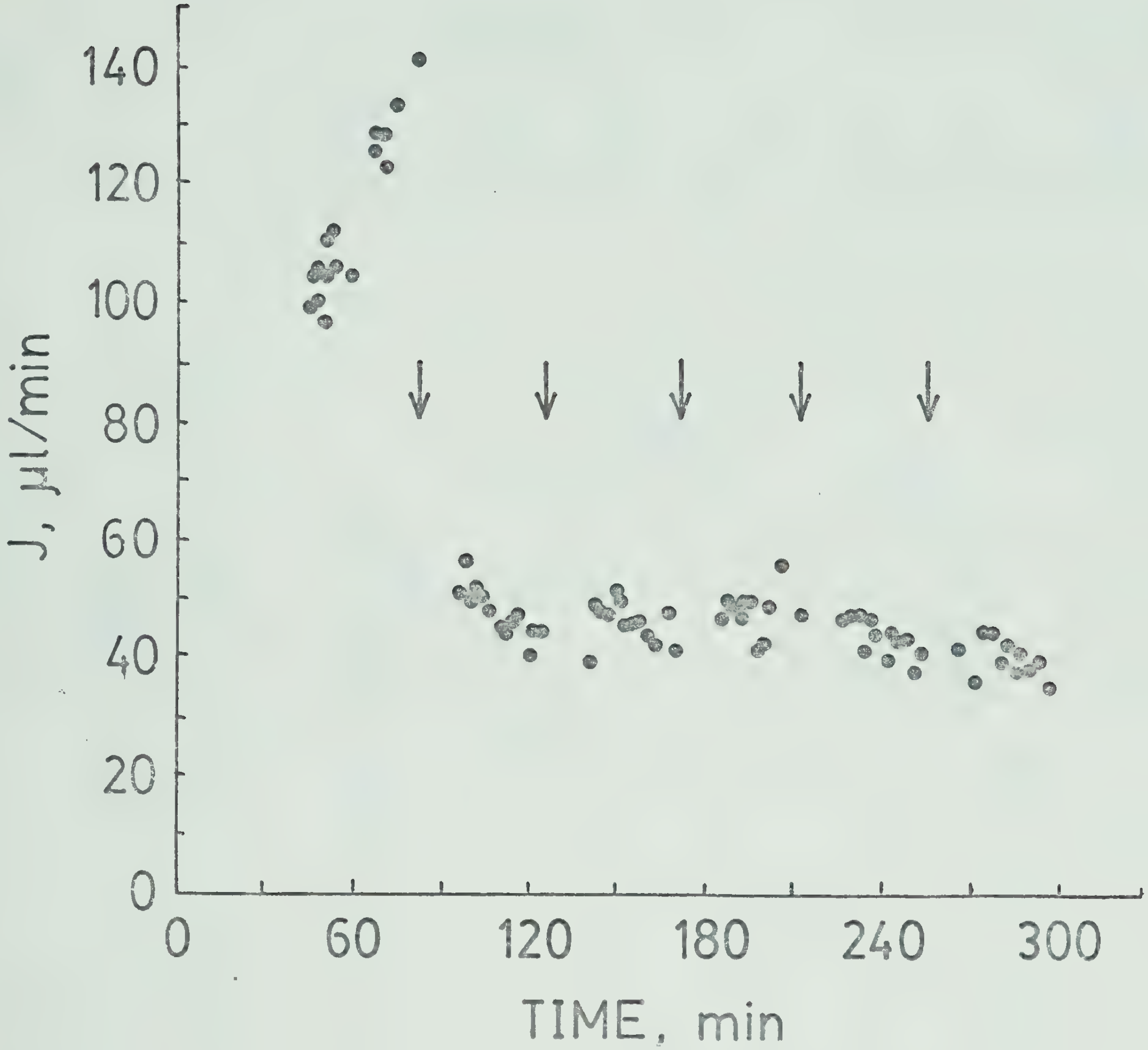

Figure 13. The influence of sucessive entire drainages and refills of the substrate solution upon water flux in a squash plant in the dark. The substrate solution was NaCl of -0.45 MPa osmotic potential. Each arrow indicates commencement of a drainage and refill.

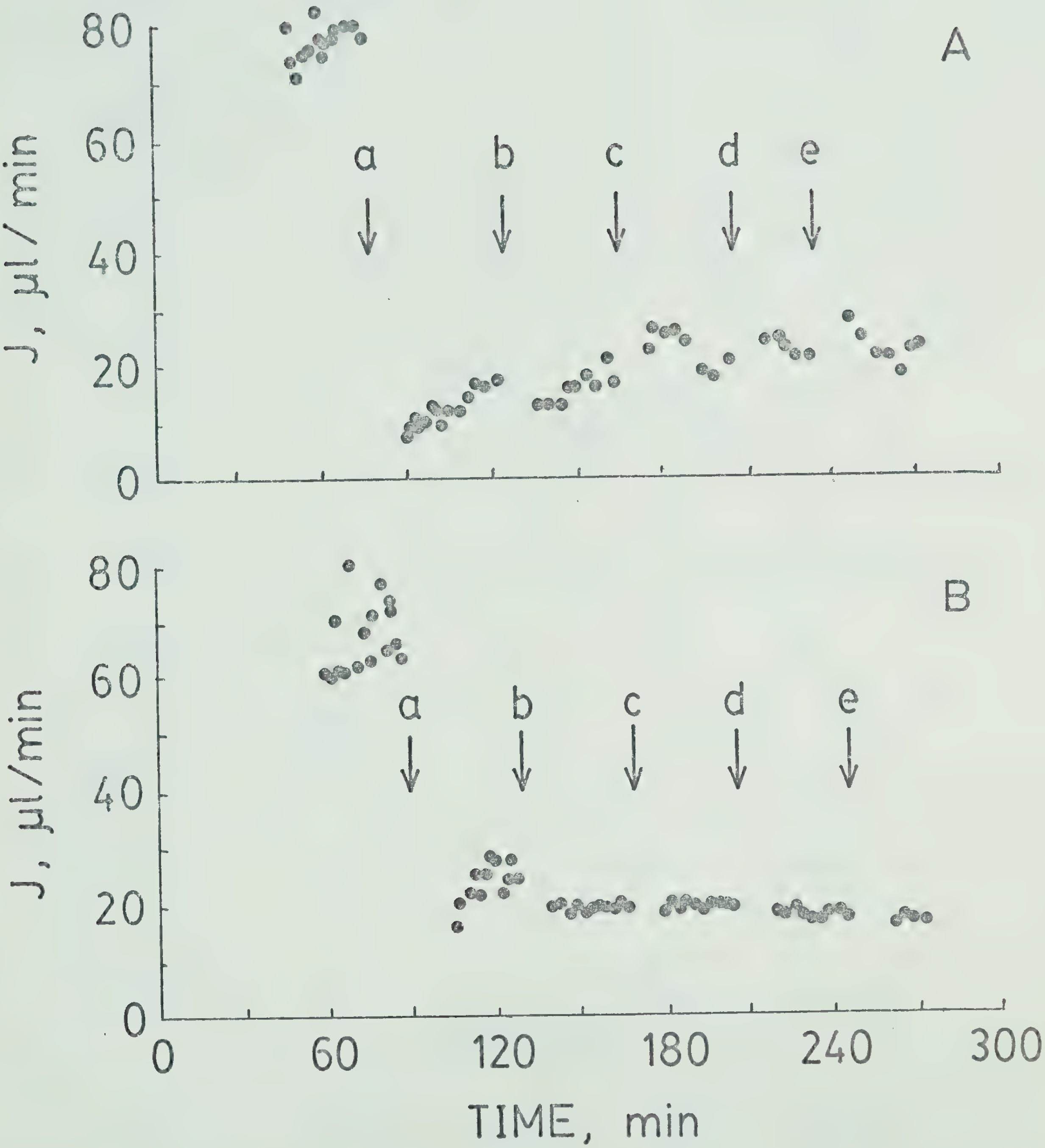

Figure 14. The influence of treating root systems of squash plants in the dark with osmotic solutions of NaCl. Initial rates are for a substrate solution of distilled water. Osmotic treatments: a) -0.5 MPa, b) -1.0 MPa, c) -2.0 MPa, d) -3.0 MPa, e) -4.0 MPa. Leaf water potential at the end of the experiment was: A) -1.1 MPa, B) -1.1 MPa.

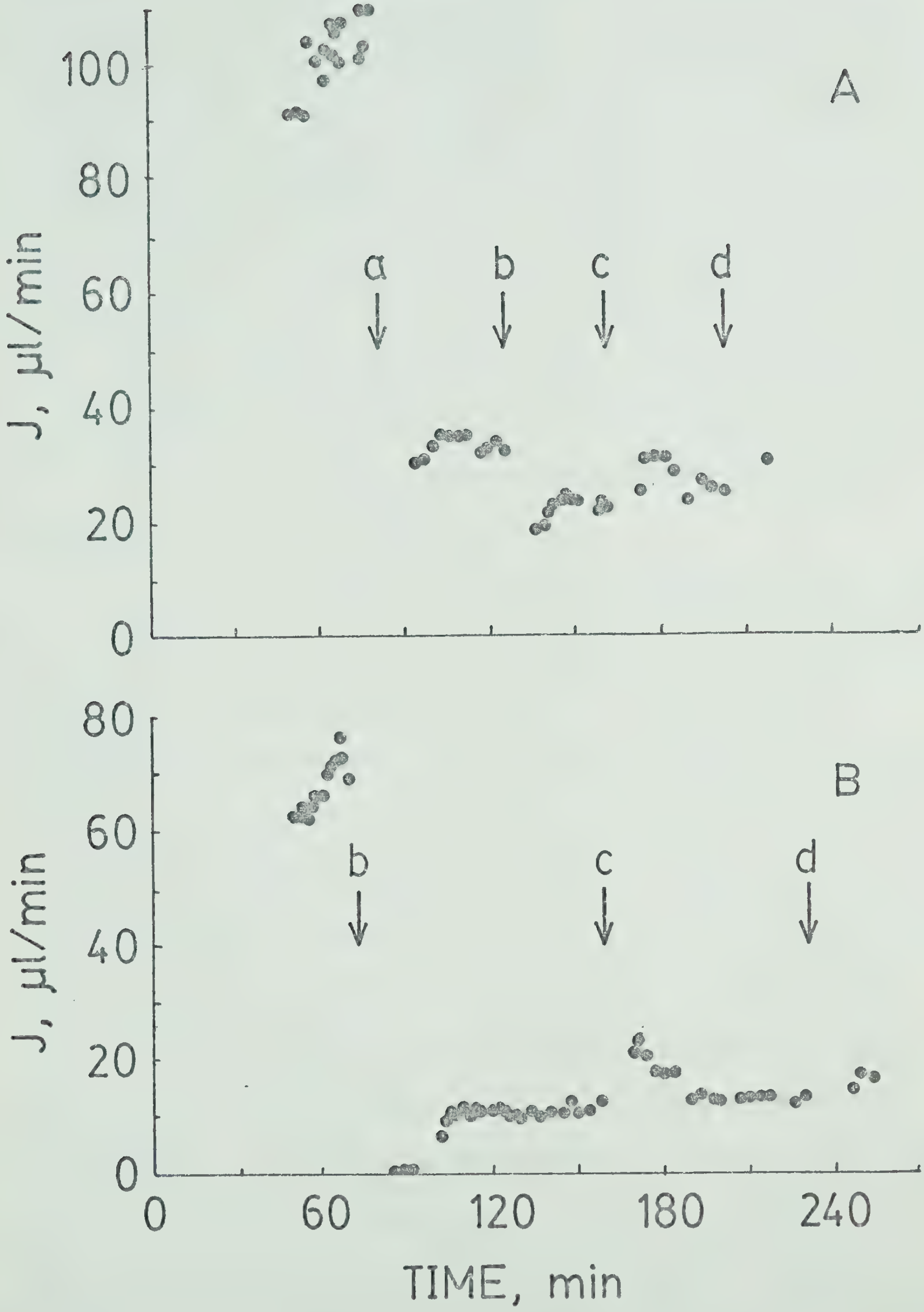

Figure 15. The influence of treating root systems of squash plants in the in the dark with osmotic solutions of NaCl. Initial rates are for a substrate solution of distilled water. Osmotic treatments: a) -0.5 MPa, b) -1.0 MPa, c) -2.0 MPa, d) -3.0 MPa. Leaf water potential at the end of the experiment was: A) -1.2 MPa, B) -1.1 MPa.

around 0.06 MPa. Leaf water potential does not equilibrate immediately with the xylem, due simply to the high resistance encountered by water moving from cells to the apoplastic pathways (Weatherly 1963), so the discrepancy between it and substrate potential was not unexpected. The value of hydrostatic pressure in the xylem must be lower than the value for the osmotic potential of the substrate if the roots are taking water up, otherwise the direction of J would be towards the roots. In one case, (fig. 15B), J was initially zero for some minutes, and then rose to the expected value and direction, indicating that equilibration between the substrate, xylem and air was occurring.

It seems unlikely that penetration of osmotica in such a short term experiment is of consequence in modifying the water potential difference from the substrate solution to the xylem water. Polyethylene glycol (PEG), mannitol, and NaCl all produce wilting in squash plants qualitatively equally, and PEG has a measurable effect on leaf water relations (Appendix A). Often questioned as an osmotic agent, NaCl has, however, been used quite successfully in similar experiments with Phaselous vulgaris (O'Leary 1969), and Millar et al. (1975) found it adequate with maize. Severe dehydration of the endodermis leading to plasmolysis has been suggested as a way in which permeability to an osmoticum is increased with water deficit. However, Levitt (1969, p. 84) has pointed out that endodermal protoplasts adhere to the transverse and radial walls, allowing no free access of osmoticum molecules into the root xylem. It appears unlikely that appreciable absorption of osmoticum by roots has occurred, and that the roots and xylem experience water

potentials approximately equal to that of the substrate salt solution.

Based upon these experiments, there is no measurable change in xylem resistance as high tensions are placed upon the xylem. These experiments do not eliminate the possibility that xylem resistance did not actually increase due to cavitation, only that in a system where cuticular resistance is the controlling factor, such changes in xylem resistance are too small to be of any consequence in having an impact on the rate of water flux. By conducting experiments with a system in which the xylem makes up a larger portion of the total resistance, any changes in xylem resistance may be detectable. The other possibility is that associated with the increase in xylem resistance, the gradient in hydrostatic pressure from the root xylem to the leaf xylem steepened, and J stayed constant as a result. As stated earlier, (p. 51) the magnitude of such a change would be rather small and difficult to measure.

It is notable that although earlier experiments showed that dehydration could affect root resistance and leaf resistance, there was apparently no change in either when the osmotic treatment was done on whole intact plants. In the case of root resistance, any changes might not have been great enough to cause an effect in J, due to the fact that in the dark, the main control of transpiration is at the cuticle (p. 51). As for the leaves, expected changes may not have occurred because leaf water potential had not greatly dropped and, as mentioned earlier (p. 45), the pathway and source of transpiration for intact and detached leaves may be different.

The very noticeable drop in J when the solutions in the root

chamber were stirred or aerated vigorously (fig. 9) was not expected. No such similar effects could be found in the literature, although Michel (1971) did find that growth of cucumber hypocotyls was affected by hormones in conjunction with stirring the substrate solution. Since the shoot was kept in the dark and received a constant treatment, the decrease in rate is explanable only by an increase in resistance somewhere in the plant due to a stimulus received at the roots. Since only extremely large changes in root or xylem resistance would be expected to reflect themselves as noticeably as in fig. 9, it appears unlikely that the change in resistance occurs at those points. Possibly further stomatal closure is stimulated to occur as shock waves from the stirring are transmitted to the leaves. Such transmissions through the xylem have been observed on several occasions (Raschke and Kühl 1969; Sheriff 1974).

SUMMARY AND CONCLUSIONS

Recent investigations, mainly in a series of papers by Milburn, have suggested that cavitation of water columns in the xylem may occur quite readily as high tensions develop. This investigation attempted to ascertain the extent of such cavitations and to determine if any appreciable changes in xylem resistance resulted. Squash, which had qualitatively been seen to have cavitating water columns, was chosen for study. Morphological as well as physiological data were collected, since the two are related.

Squash xylem has a very low resistivity to water flux, owing to a number of vessels of very large radius and a high efficiency of water conduction. However, since its vessels are quite large, cavitation may potentially occur with ease. Even so, many smaller conduits, which are more resistant to cavitation, are present and may ensure a supply of water to the leaves.

In order to measure changes in xylem resistance, any end effects, or changes in resistance of the root system or the leaves had to be determined. Root resistance was found to increase with dehydration, probably due to changes in the pathway taken by water. Much of the variation in leaf resistance was avoided by keeping the plants in the dark. However, dehydration did cause a further increase in leaf resistance either due to additional stomatal closure or cuticular drying, or both.

While controlling the driving force for water flux in intact squash plants, two treatments were undertaken to search for changes

in overall resistance beyond those expected due to roots and leaves. The first treatment definitely changed xylem resistance by actually cutting xylem conduits. Although such a treatment undoubtedly did increase xylem resistance, rates of transpiration remained constant, suggesting no resistance change. Quite probably, the change in resistance was too small to be measured with the experimental design, which allowed only very large changes in resistance to be detected. Alternatively, the gradient of hydrostatic pressure along the xylem may have increased, even though the total driving force for water movement remained constant.

The second treatment attempted to stimulate cavitation by imposing high xylem tensions over the entire plant. This method could conceivably bring about cavitations anywhere in the plant, rather than only at one place as did the cutting experiments. Even when the roots were treated with osmotic solutions of -4.0 MPa, there was no detectable change in xylem resistance, indicating that cavitation had not occurred to a large enough extent to have a measurable change in water flux. However, if xylem resistance had contributed proportionately more of the total resistance, the measurement would have been more sensitive and any such cavitations might have been apparent.

A plant which might naturally experience high xylem tensions in field conditions would be expected to show no apparent change in xylem resistance. This is because its stomata would be closed due to water deficit. Its rate of cuticular transpiration would be normally maintained and changes in the resistance of the root or xylem would not play a role in the control of J. However, if the water deficit was relieved so that stomata could open, an increase

in xylem resistance may be apparent either as a decrease in leaf water potential, lower rates of water flux, or both. This point was illustrated by Boyer (1971b) who dehydrated 2 sunflower plants to produce cavitations and then removed embolisms from one by a vacuum treatment. Upon measuring recovery of leaf water potential, photosynthesis, and transpiration, the plant from which embolisms had been removed recovered much more quickly.

Cavitation, although possibly not initially apparent, may be critical during a post-stress recovery period. In the case of many species, root pressure may occur with relief of water deficit and aid in the process of refilling cavitated elements. Squash can develop root pressures of nearly 0.04 MPa, enough to push water as high as the plant grows.

Milburn's series of papers suggested that cavitation occurs readily. If each "click" which he detects is a cavitation, then even hundreds of "clicks" may not indicate cavitation of a large portion of tracheary elements. Zimmermann (1976, p. 229) found that surprisingly large numbers of vessels are present in Ricinus petioles, and suggested that a few cavitations would have little effect. It appears that a very large number of cavitations would need to occur before the xylem would become sufficiently disabled to show a change in its apparent resistance. Another problem with much of Milburn's work is that, often, leaves in which "clicks" were detected had been excised and air may have entered the xylem, stimulating more cavitations as West and Gaff (1976) suggested.

It seems then that cavitation certainly does occur in plants regularly, and that such events are stimulated by tensions which

develop in the xylem as a result of low soil moisture and high atmospheric demand for water. However, there will probably be no detectable changes in the apparent resistance of the xylem. Either the increase is simply too small, or hydrostatic pressure gradients steepen to re-elevate the flow rate, but any such steepening is too small to be detectable.

Certainly some of the morphological features of the xylem may be considered to result from selection to cope with cavitations. Short and narrow vessels, and many of them, are most important. It is also interesting that the xylem, in which all long distance transport of water occurs, is also the most efficient of all water pathways in terms of the pressure gradients required. It is important that this be so, simply because the leaf water potential need not be as low in order to supply the proper driving force. Consequently, leaf physiological processes will occur in a more highly hydrated medium. Clearly, the xylem is a tissue which evolved both an efficient and effective mechanism and, as well, allowed for safety and maintainence.

LITERATURE CITED

Aston, M.J., and M.M. Jones. 1976. A study of the transpiration surfaces of Avena sterilis L. var. Algerian leaves using monosilicic acid as a tracer for water movement. Planta 130: 121-130.

Boyer, J.S. 1969. Free energy transfer in plants. Sci. 163: 1219-1220.

Boyer, J.S. 1971a. Resistances to water transport in soybean, bean, and sunflower. Crop Sci. 11: 403-407.

Boyer, J.S. 1971b. Recovery of photosynthesis in sunflower after a period of low leaf water potential. Plant Physiol. 47: 816-820.

Briggs, L.J. 1950. Limiting negative pressure of water. J. Appl. Physics. 21: 721-722.

Brouwer, R. 1953. Water absorption by the roots of Vicia faba at various transpiration strengths. I. Analysis of the uptake and the factors determining it. Proc. Kon. Ned. Akad. Wet. C56: 106-115.

Budgett, H.M. 1912. The adherence of flat surfaces. Proc. Roy. Soc. (Lond) A 86: 25-35.

Burbano, J.L., T.D. Pizzolato, P.R. Morey, and J.D. Berlin. 1976. An application of the prussian blue technique to a light microscope study of water movement in transpiring leaves of cotton (Gossypium hirsutum L.). J. Expt. Bot. 27: 134-144.

Byott, G.S., and D.W. Sheriff. 1976. Water movement into and through Tradescantia virginiana L. leaves. II. Liquid flow pathways and evaporation sites. J. Expt. Bot. 27: 634-639.

Byrne, G.F., J.E. Begg, and G.K. Hansen. 1977. Cavitation and resistance to water flow in plant roots. Agric. Meteorol. 18: 21-25.

Carlquist, S. 1975. Ecological strategies of xylem evolution. Univ. of California Press, Berkeley. 259 pp.

Chung, H. and P.J. Kramer. 1975. Absorption of water and ^{32}P through suberized and unsuberized roots of loblolly pine. Can. J. For. Res. 5: 229-235.

Cox, L.M., and L. Boersma. 1967. Transpiration as a function of soil temperature and soil water status. Plant Physiol. 42: 550-556.

Crafts, A.S. 1939. Solute transport in plants. Sci. 90: 337-338.

Crafts, A.S., H.B. Currier, and C.R. Stocking. 1949. Water in the physiology of plants. Chronica Botanica Co., Waltham, Mass. 240 pp.

Dalton, F.N., P.A.C. Raats, and W.R. Gardner. 1975. Simultaneous uptake of water and solutes by plant roots. Agron. J. 67: 334-339.

Diamond, A.E. 1966. Pressure and flow relations in vascular bundles of the tomato plant. Plant Physiol. 41: 119-131.

Dixon, H.H. 1914. Transpiration and the ascent of sap in plants. MacMillan, London. 216 pp.

Dubé, P.A., K.R. Stevenson, G.W. Thurtell and R.B. Hunter. 1975. Effects of water stress on leaf respiration, transpiration rates in the dark, and cuticular resistance to water vapor diffusion of two corn hybirds. Can. J. Plant Sci. 55: 565-572.

Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, New York. 412 pp.

Esau, K. 1965. Plant anatomy. John Wiley and Sons, New York. 767 pp.

Fiscus, E.L. 1975. The interaction between osmotic- and pressure-induced water flow in plant roots. Plant Physiol. 55: 917-922.

Fisher, J.C. 1948. The fracture of liquids. J. Appl. Physics. 19: 1062-1067.

Greenridge, K.N.H. 1955. Studies in the physiology of forest trees. II. Experimental studies of fracture of stretched water columns in transpiring trees. Amer.J. Bot. 42: 28-37.

Greenridge, K.N.H. 1957. Ascent of sap. Ann. Rev. Plant Physiol. 8: 237-256.

Haider, K. 1954. Zur morphologie vnd physiologie der sporangien leptosporangiater farne. Planta 44: 370-411.

Hammel, H.T. 1967. Freezing of xylem sap without cavitation. Plant Physiol. 42: 55-66.

Hayward, A.T.J. 1971. Negative pressure in liquids: can it be harnessed to serve man? Am. Scientist 59: 434-443.

Hayward, H.E. 1938. The structure of economic plants. Macmillan, New York. 674 pp.

Holmgren, P., P.G. Jarvis, and M.S. Jarvis. 1965. Resistances to carbon dioxide and water vapor transfer in leaves of different plant species. Physiol. Plant. 18: 557-573.

Hsiao, T.C. 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 10: 519-570.

Ivanov, L.A. 1928. Zur methodik der transpirations bestimmung am standort. Ber. Deut. Bot. Ges. 46: 306-310.

Jarvis, P.G. 1975. Water transfer in plants. In: Heat and mass transfer in the plant environment. Part 1. Transfer processes in the plant environment. Edited by: D.A. de Vries and N.H. Afgan. Scripta Book Co., Washington, D.C. pp. 369-394.

Jarvis, P.G., and R.O. Slatyer. 1970. The role of the mesophyll cell wall in leaf transpiration. Planta 90: 303-322.

Jensen, R.D., S.A. Taylor, and H.H. Wiebe. 1961. Negative transport and resistance to water flow through plants. Plant Physiol. 36: 633-638.

Jensen, W.A. 1962. Botanical Histochemistry. W.H. Freeman, San Franciso. 408 pp.

Kendrick, F.B., K.L. Wismer, and K.S. Wyatt. 1924. Supersaturation of gases in liquids. J. Phys. Chem. 28: 1308-1315.

King, A.L. 1944. The spore discharge mechanism of common ferns. Proc. Natl. Acad. Sci. (U.S.) 30: 155-161.

Kramer, P.J. 1949. Plant and soil water relationships. McGraw-Hill, New York. 347 pp.

Kuiper, F., and P.J.C. Kuiper. 1974. Permeability and self-induction as factors in water transport bean roots. Physiol. Plant. 31: 159-162.

Lang, A.R.G. 1967. Osmotic coefficient of water potentials of sodium chloride solutions from 0-40°C. Aust. J. Chem. 20: 2017-2023.

Lawlor, D.W. 1970. Absorption of polyethylene glycols by plants and their effects on plant growth. New Phytol. 69: 501-513.

Levitt, J. 1969. Introduction to plant physiology. C.V. Mosby, St. Louis. 304 pp.

Loomis, W.E., P.R. Santamaria, and R.S. Gage. 1960. Cohesion of water in plants. Plant. Physiol. 35: 300-306.

Mackay, J.F.G., and P.E. Weatherly. 1973. The effects of transverse cuts through the stems of transpiring woody plants on water transfer and stress in leaves. J. Expt. Bot. 24: 15-28.

Mayo, J.M. 1974. A thermocouple psychrometer chamber for field and laboratory use. Can. J. Plant Sci. 54: 597-598.

Meidner, H. 1975. Water supply, evaporation, and vapor diffusion in leaves. J. Expt. Bot. 26: 666-673.

Meidner, H. 1976. Water vapor loss from a physical model of a substomatal cavity. J. Expt. Bot. 27: 691-694.

Meidner, H., and D.W. Sheriff. 1976. Water and plants. John Wiley and Sons, New York. 148 pp.

Michel, B.E. 1971. Further comparisons between carbowax 6000 and mannitol as suppressants of cucumber hypocotyl elongation. Plant Physiol. 48: 513-516.

Milburn, J.A. 1973a. Cavitation in Ricinus by acoustic detection: induction in excised leaves by various factors. Planta 110: 253-265.

Milburn, J.A. 1973b. Cavitation studies on whole Ricinus plants by acoustic detection. Planta 112: 333-342.

Milburn, J.A., and R.P.C. Johnson. 1966. The conduction of sap. II. Detection of vibrations produced by sap cavitation in Ricinus xylem. Planta 69: 43-52.

Milburn, J.A., and M.E. Mclaughlin. 1974. Studies of cavitation in isolated vascular bundles and whole leaves of Plantago major L. New Phytol. 73: 861-871.

Millar, A.A., W.R. Gardner, and S.M. Golz. 1971. Internal water status and water transport in seed onion plants. Agron. J. 63: 779-784.

Moreshet, S. 1970. Effect of environmental factors on cuticular transpiration resistance. Plant Physiol. 46: 815-818.

Morrow, P.A., and R.O. Slatyer. 1971. Leaf resistance measurements with diffusion porometers: precautions in calibration and use. Agric. Meteorol. 8: 223-233.

Newman, E.I. 1969. Resistance to water flow in the soil and plant. II. A review of experimental evidence on the rhizosphere resistance. J. Appl. Ecol. 6: 261-272.

Newman, E.I. 1976. Water movement through root system. Phil. Trans. R. Soc. (Lond) Ser. B, 273: 433-613.

Newman, F.H., and V.H.L. Searle. 1948. The general properties of matter. 4th ed. Edward Arnold and Co., London. 431 pp.

Nobel, P.S. 1970. Introduction to biophysical plant physiology. W.H. Freeman, San Franciso. 488 pp.

O'Leary, J.W. 1969. The effect of salinity on permeability of roots to water. Israel J. Bot. 18: 1-9.

Ordin, L., and S. Gairon. 1961. Diffusion of tritiated water into roots as influenced by water status of tissue. Plant Physiol. 36: 331-335.

Parsons, E.R., and P.J. Kramer. 1974. Diurnal cycling of root resistance to water movement. Physiol. Plant 30: 19-23.

Pizzolato, T.D., J.L. Burbano, J.D. Berlin, P.A. Morey, and R.W. Pease. 1976. An electron microscope study of the path of water movement in transpiring leaves of cotton (*Gossypium hirsutum* L.). J. Expt. Bot. 27: 145-161.

Preston, R.D. 1938. The contents of the vessels of *Fraxinus americana* L. with respect to the ascent of sap. Ann. Bot. 2: 1-22.

Raschke, K., and U. Kuhl. 1969. Stomatal responses to changes in atmospheric humidity and water supply: Experiments with leaf sections of *Zea mays* in CO_2-free air. Planta 87: 36-48.

Richter, H. 1973. Frictional potential losses and total water potential in plants: a re-evaluation. J. Expt. Bot. 24: 983-994.

Scholander, P.F., W.E. Love, and J.W. Kanwisher. 1955. The rise of sap in tall grapevines. Plant Physiol. 30: 93-104.

Schönherr, J. 1976. Water permeability of cuticular membranes. *In*: Water and plant life. *Edited by*: Lange, O.L., L. Kappen and E. -D. Schulze. Springer Verlag, Berlin, New York. pp. 148-159.

Scott, A.F., D.P. Shoemaker, K.N. Tanner and J.G. Wendel. 1948. A study of the Berthelot method for determining the tensile strength of a liquid. J. Chem. Phys. 16: 495-502.

Sheriff, D.W. 1974. A model of plant hydraulics under non-equlibrium conditions. I. Stems. J. Expt. Bot. 25: 552-561.

Slatyer, R.O. 1967. Plant - water relationships. Academic Press, London, New York. 366 pp.

Slavik, B. 1974. Methods of studying plant water relations. Springer Verlag, New York. 449 pp.

Stocking, C.R. 1943. Continuity, tensions, and redistribution of water in plants. Ph.D. Dissert. Univ. of Calif.

Temperely, H.N.V. 1946. The behavior of water under hydrostatic tension: II. Proc. Phys. Soc. (London). 58: 436-443.

Temperely, H.N.V. 1947. The behavior of water under hydrostatic tension: III. Proc. Phys. Soc. (London). 59: 199-208.

Van den Honert, T.H. 1948. Water transport in plants as a catenary process. Disc. Faraday Soc. 3: 146-153.

Weatherly, P.E. 1963. The pathway of water movement. *In*: The water relations of plants. *Edited by*: Rutter, A.J., and F.H. Whitehead. Blackwell Scientific Publications, London.

West, D.W., and D.F. Gaff. 1976. Xylem cavitation in excised leaves of *Malus sylvestris*. Mill and measurement of leaf water status with the pressure chamber. Planta 129: 15-18.

Williams, W.T., and F.A. Amer. 1957. Transpiration from wilting leaves. J. Expt. Bot. 8: 1-19.

Zimmermann, M.H. 1971. Transport in the xylem. *In*: Zimmermann, M.H., and C.L. Brown. Trees: structure and function. Springer-Verlag, Berlin, New York. pp. 169-220.

Zimmermann, M.H. 1976. A study of vascular patterns in higher plants. *In*: Transport and transfer processes in plants. *Edited by*: Wardlaw, I.F., and J.B. Passioura. Academic Press, New York. 484 pp.

APPENDIX A

APPENDIX A

WATER RELATIONS

Purpose

This experiment was designed to gain familiarity with the characteristics of water relations of squash and look for any gross indication of cavitation having occurred, such as a large difference in water relations between pre- and post-stress periods. Since the data are only exploratory and comparative, it was not deemed necessary to conduct the experiment several times.

Methods and Materials

Response of leaf water potential (ψ_1), leaf resistance (R_1), and transpiration (J) were studied on plants which had been in solution culture for about 4 weeks. As for other plants in the course of this work, the two plants upon which measurements were made were brought to the growth chamber several days before the experiment began.

Light in the chamber was supplied from fluorescent and incandescent bulbs to provide 180μE/m^2-s at the top of the plants. Temperature was maintained at $22 \pm 1^{\circ}$C and monitored with the growth chamber recorder and a Hg-glass thermometer. Relative humidity was maintained at one of three levels ±5% and monitored with the growth chamber instrumentation and Assman aspirated wet and dry bulb thermometers. Both leaf and air temperature were measured with 0.076 mm. copper-constantan wire thermocouples. Output from the thermocouples was measured with a microvoltmeter (model 845 A B, Fluke, Seattle, Washington).

Transpiration was measured gravimetrically on a 20 kg capacity triple beam balance (Ohaus Scale Corporation, Union, New Jersey) to ± 1 g. Since the nutrient solutions were constantly aerated, a control pot identical to the others but containing no plant was used to correct for evaporation from the pots. Transpiration rate was calculated on a leaf area basis. Since some death of leaves occurred with high stresses, leaf area was slightly smaller during the second half of the experiment. Leaf area was determined by tracing the leaves on paper, and multiplying the weight of the trace by a calibration factor.

Experiments began about 1 h after the chamber lights came on. In order to prevent oscillations in transpiration in response to large abrupt changes in evaporative demand, relative humidity was lowered in a stepwise manner over a 2 hr period. The pots were then weighed, and after 2 h leaf water potential samples were taken and leaf resistance measured. Next, the pots were re-weighed and then relative humidity lowered during another 2 h period. The sampling procedure was then repeated. Three levels of relative humidity were attained in the course of an experiment: 90, 55 and 40%, and vapor pressure difference (VPD) between the leaves and the air calculated for presentation.

The leaf water potential of 5 mm diameter discs was determined with Spanner-type thermocouple psychrometers built from the design of Mayo (1975). Calibration was done with KCl solutions of known osmotic potential. Leaf tissue samples were allowed to equilibrate for 2.5 h in a 22°C water bath and then read with the microvoltmeter. Two samples were taken from the margin of mature leaves.
Only one leaf was sampled during an experiment at any given substrate

potential.

Leaf resistance, also measured at the leaf margins, was determined with a Lambda Instruments diffusion porometer (Lincoln, Nebraska). Care was taken to calibrate and use the sensor under procedures given by Morrow and Slayter (1971). Since squash is amphistomatous, R_l was measured on both sides of the leaf, and the overall resistance then calculated.

Dehydration and rehydration was attained by treating the plants with Hoagland's solution containing various amounts of polyethylene glycol-6000 (PEG-6000). A calibration curve of osmotic potential and weight of PEG-6000 dissolved in water was constructed and found to be identical to that of Lawlor (1970). Initial data were collected for Hoagland's solution only (osmotic potential = -0.07 MPa), then the plant irrigated in a solution of Hoagland's solution plus enough PEG-6000 to give an osmotic potential of -0.7 MPa. The plant was irrigated with a new solution at the end of a day's measurements so that sampling could proceed without delay the next morning. Additional treatments with -1.2, -0.7, -0.7 and -0.07 (Hoagland's only) MPa solutions followed on successive days, except, between treatments of -0.7 and -1.2 MPa respectively, where growth chamber repairs forced a one day delay.

Results

Two plants were used in the experiment; however since the two plants showed the same responses, and since data were incomplete for one plant, Table 4 only shows data from one plant. The minimum leaf water potential was -0.34 MPa, as noted for VPD = 0.55 KPa and a substrate potential of -0.07 MPa. As can be seen, ψ_l

Table 4. Effect of substrate potential and vapor pressure difference (VPD) on water flux (J), leaf resistance (R_1), and leaf water potential (ψ_1) in squash.

Substrate Potential (MPa)	VPD* (kPa)	J (ul/dm^2-min)	R_1 (sec/cm)	ψ_1 (MPa)
-0.07	0.55	13.6	1.6	-0.34
	1.23	20.0	6.3	-0.41
	1.75	18.1	6.5	-0.63
-0.70	0.32	4.2	9.0	-0.50
	1.42	5.7	8.8	-0.80
	1.91	10.6	8.4	-0.77
-1.20	0.66	4.2	20.3	-0.96
	1.36	1.1	24.6	-1.08
	2.03	2.3	21.2	-1.19
-0.70	0.41	4.3	6.7	-0.75
	1.08	3.9	11.0	-0.79
	1.55	3.1	8.1	-0.74
-0.70	0.20	1.9	6.0	-0.62
	1.12	5.8	6.0	-0.75
	1.91	4.5	4.6	-0.83
-0.07	0.10	7.4	3.1	-0.43
	1.17	9.3	8.7	-0.72

* 1 kPa = 10 mbar.

decreased in a stepwise fashion as dehydration occurred, and then increased as rehydration occurred. However, ψ_1 actually remained greater than ψ_s when either -0.70 or -0.12 MPa substrate potential treatments were given.

Leaf resistance reached a minimum of 1.6 sec/cm while the plant was irrigated in Hoagland's solution only and atmospheric demand was low. In nearly every treatment, leaf resistance increased as atmospheric demand increased. With decreasing substrate potentials, leaf resistance increased, reaching a maximum of nearly 25 s/cm at a substrate potential of -1.2 MPa and high evaporative demand. A great deal of variation in successive measures of R_1 was found, even along a single leaf, with adaxial measures ±13 s/cm, and abaxial determinations ± 2 s/cm. Transpiration rate was highest on the initial day when the substrate was Hoagland's solution only. As dehydration proceeded to -1.2 MPa substrate potential, transpiration dropped to a value only one-tenth to one-twentieth of its highest value, reflecting the increase in leaf resistance. During rehydration, transpiration was depressed below that of the dehydration levels, even when comparison was made between identical substrate potentials and similar atmospheric demands.

Conclusions

Generally, these results show that squash reacts to water stress in a manner similar to that of many other species. Leaf water potential decreased, leaf resistance increased, and transpiration decreased. It is notable that the measured leaf water potential

was not always as low as the substrate osmotic potential. Quite likely, the inaccuracy of the thermocouple psychrometers is responsible for this. There may be a distinct relationship between leaf resistance and relative humidity as has been suggested for other species recently. Apparently, water stress had an effect on the stomata, possibly mediated through abscisic acid, for leaf resistance remained comparatively higher during the post-stress period. As a result the post-stress transpiration rate was only about one-half of the pre-stress rate. Since leaf resistances adequately explain the change in transpiration rate, and leaf water potential was about the same in pre- and post-stress data, there is not any indication of cavitation having occurred.

APPENDIX B

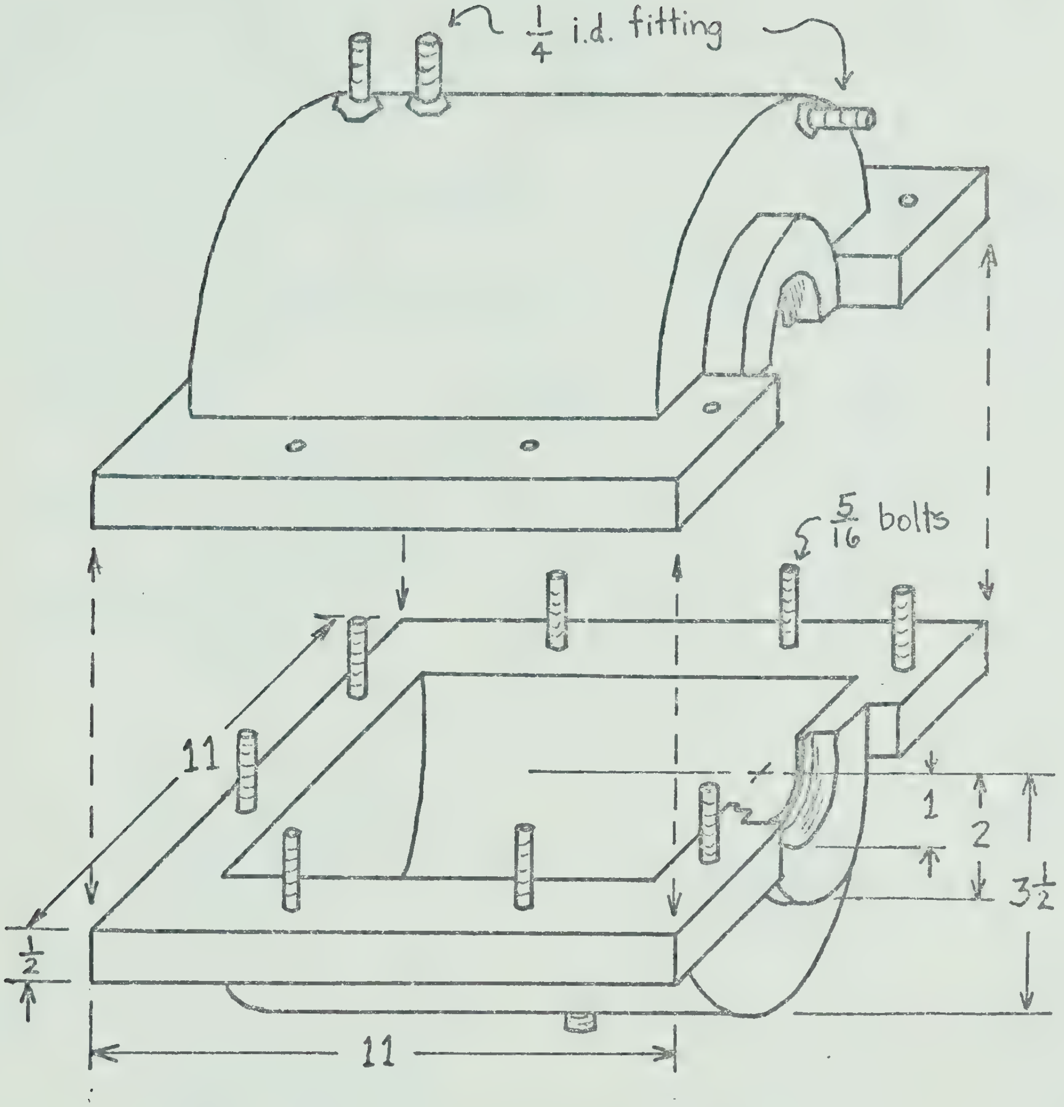

Figure 16. Diagram of the root chamber section of the potometer used in this study. All dimensions are in inches. The chamber was machined from 1/2 inch plexiglass. Fittings which were used for measurements or drainages and refillings were located on the top, bottom, and side. The stem and sealing inserts go through the threaded hole on the end. See fig. 17 for a diagram of the sealing inserts and threaded pressure tightener. Not shown are plexiglass supports on the bottom half of the root chamber.

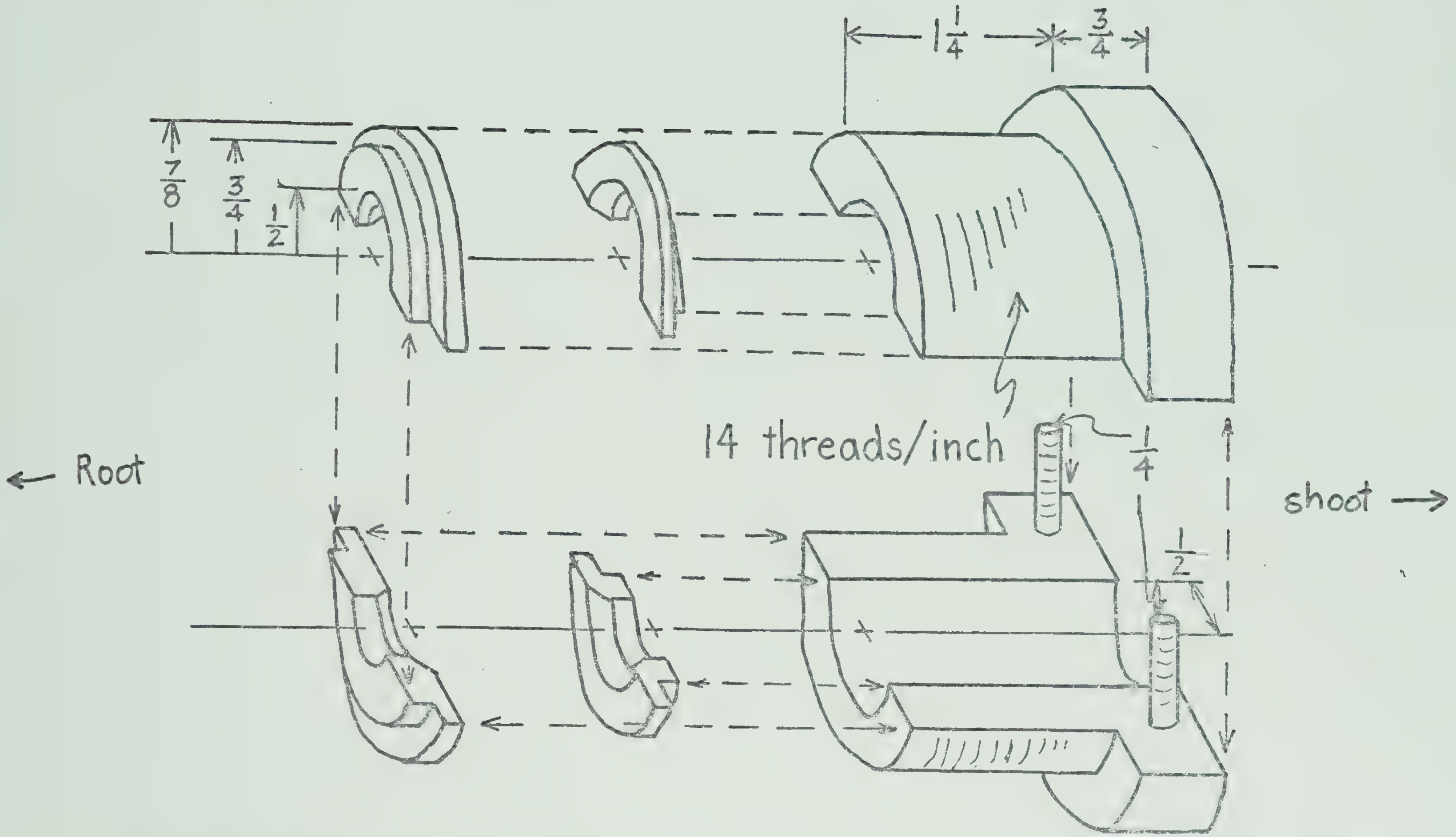

Figure 17. Sealing inserts and threaded pressure tightener for sealing plants in the root chamber. All dimensions are in inches. All materials were plexiglass except the steel 1/4 inch dowells holding the 2 halves of the pressure tightener together. The stem of the plant followed the center line in the directions shown. Terostat was used to seal between the inserts and along the plant stem.

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